THE UNIVERSITY OF ALBERTA

VARYING LEVELS OF ENERGY AND ROUGHAGE IN RATIONS FOR LACTATING DAIRY COWS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Varying levels of energy and roughage in rations for lactating dairy cows" submitted by Arie Leonardus Hoogendoorn, B.Sc. (Agr.), M.Sc., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Increasing energy from 90 to 100 or 120 per cent of the calculated requirement for digestible energy, and increasing the quantity of roughage in the ration from 0.75 to 1.50 or 2.50 kg per 100 kg of body weight, did not appreciably or consistently affect dry matter or gross energy consumption by Holstein-Friesian cows early in the lactation at maximum milk production (period 1). During this period, all cows were eating to full capacity, which appeared to be approximately 90 per cent of their calculated requirements for digestible energy. Near the end of lactation (period 2), dry matter and gross energy intake by the cows increased as the level of energy or roughage increased in the daily ration.

There were no significant differences detected between apparent digestion coefficients of dry matter, crude protein or gross energy within either period, but the apparent digestibility of the ration was significantly lower in period 2 than in period 1.

On the average, molar concentrations of total volatile fatty acids in rumen liquid were lower and the pH was higher in the second period than in the first period. Level of energy in the ration did not affect the molar proportions of acetate, propionate, n-butyrate and n-valerate, but did have a significant effect on the proportions of iso-butyrate and iso-valerate. An increase in the proportion of roughage in the ration was associated with a significant increase in the molar proportion of acetate and a decrease in the proportion of propionate.

Although the level of energy or roughage fed did not have significant effects on the 28-week average milk production by each group, a significant decrease was obtained after the 16th week for cows fed the low level of energy as compared to cows fed the medium and high energy rations. Percentage

milk fat was significantly higher when cows were fed the rations with high proportions of roughage; percentages of milk protein and solids-not-fat were significantly higher when cows were fed rations with the higher levels of energy.

The major components of butterfat were 14:0, 16:0, 18:0 and 18:1 fatty acids with average percentages between 11.4 and 29.0 of the total fatty acids measured. The 4:0, 6:0, 8:0, 10:0, 12:0, 18:2 and 18:3 fatty acids were present in amounts varying from 1.3 to 4.4 per cent. The higher levels of energy in the ration were associated with significant increases in the 8:0, 10:0, 12:0 and 14:0 acids, and significant decreases in the 18:0, 18:1 and 18:3 fatty acids. In general, there was a greater incorporation of long chain fatty acids into milk fat during the first half of lactation, associated with loss in body weight. An increase in the level of roughage in the ration significantly increased the 4:0, 6:0 and 18:3 acids and significantly decreased the 18:2 acid.

The average data for the two periods showed that cows fed the low and medium levels of energy produced milk more efficiently, in terms of the digestible energy consumed above maintenance per kg of fat-corrected milk, than cows fed the high energy ration. Energy consumed per unit of milk produced increased with each increase in level of roughage in the ration. The average net energetic efficiency for the two periods was similar for the cows fed energy at 90 or 100 per cent of their requirements, and this was significantly better than that for cows fed energy at 120 per cent. Average net energetic efficiency for milk production decreased as the proportion of roughage in the ration increased.

It appeared that maximum milk production could be obtained by feeding rations to provide digestible energy at 90 per cent of calculated

requirements during the peak of lactation. As production began to decline, the daily ration decreased to the extent that the cows could consume a ration providing energy up to 100 per cent of their requirements. This increase in the level of feeding appeared necessary to maintain persistency in the lactation.

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INTRODUCTION

Until recently the feeding of lactating dairy cows was based on practices and standards derived from early research with cows of comparatively low potential for milk production. During the last quarter of a century, levels of milk production have increased substantially; recent feeding trials have demonstrated that production in many herds can be increased further by providing additional feed early in the lactation.

In the past few years, it has been shown that energy intake by cows can be increased, primarily by offering cereal grains at levels which were formerly considered undesirable and even hazardous to the health of the animal. At the same time, there has been increasing awareness of the need to re-evaluate nutrient requirements for milk production at the high levels that are now attained in many herds. The most recent publication by the National Research Council on Nutrient Requirements of Dairy Cattle has attempted to indicate levels of energy adequate at all levels of production, and has taken into consideration the fact that requirements per unit of milk vary at different levels of production.

Much of the recent research has been of short duration and conducted during periods in the last two-thirds of the lactation, when production and feed consumption are declining. In many instances, the composition of the experimental rations has been of limited practical application to conditions in Western Canada.

In numerous reports, it has been pointed out that rations of different composition, particularly those with high proportions of concentrate, may have considerable effects on fermentation processes in the rumen. Changes in the end-products of digestion affect the availability of specific metabolites for maintenance and milk production, and may have considerable influence on total

milk production and its composition.

Consequently, an experiment was undertaken to study rations containing widely varying proportions of roughage and concentrate common to Western Canadian conditions and fed at different levels of energy. Their effects were measured on feed intake, digestibility, rumen fermentation, milk production and composition, and energetic efficiency of cows during a complete lactation.

To simplify digestion studies with the number of animals involved in this experiment, an initial study was conducted to assess the suitability of chromic oxide as a reference material in estimating digestion coefficients.

REVIEW OF LITERATURE

The use of chromic oxide in determination of digestibility

The determination of digestibility of a dietary nutrient requires measurements of the quantity of the nutrient consumed by the animal and excreted in the feces over a period of time. The conventional method is time consuming and laborious, particularly with large animals, since it involves total collection of the feces, uncontaminated by urine, for a period of approximately five days. Consequently, considerable attention has been directed to the use of indicator techniques which enable estimation of fecal excretion from a few small samples, thus eliminating the need for total collection of feces in the determination of digestibility. The major requirement of the indicator technique is a substance which is completely indigestible, uniformly blended with the feces and fully recoverable by chemical analyses (Maynard and Loosli, 1962).

Chromic oxide (Cr_2O_3) is a readily available material that has the properties required for indicator techniques, and has been used successfully in digestion studies with monogastric species (Clawson, et al., 1955; Raymond and Minson, 1955). However, Cr_2O_3 as an indicator in digestion studies with ruminants has provided variable results. It cannot be mixed uniformly into roughage rations and does not pass out of the rumen uniformly with the rumen contents (Corbett et al., 1959). Consequently in digestion trials with ruminants, it has been difficult to obtain representative fecal samples which would permit close and consistent agreement between digestion coefficients determined with the indicator and conventional methods (Barnicoat, 1945; Brisson et al., 1957; Hardison and Reid, 1953; Kane et al., 1950; Lassiter et al., 1966; McGuire et al., 1966).

Attempts to obtain uniform passage of Cr_2O_3 from the rumen by frequent

administration in capsule form, or through incorporation of the Cr_2O_3 into sustained release pellets with plaster of Paris have not been completely successful (Brisson and Pigden, 1958; Corbett et al., 1960; Fisher et al., 1965; Pigden and Brisson, 1957; Pigden et al., 1964; Troelsen, 1961).

Cr_2O_3 has been used successfully as an indicator in ruminant digestion studies when it was absorbed onto paper. Ingestion of measured quantities of this material, which appears to disintegrate slowly in the rumen, results in a relatively uniform concentration of Cr_2O_3 in fecal samples (Corbett et al., 1960; Cowlishaw and Alder, 1963; Edin, 1926; Johnson et al., 1964; Langlands et al., 1963 a,b).

Troelsen (1963) pelleted paper containing Cr_2O_3, to obtain a product which could be administered easily in the required amount daily in digestion studies with cattle and sheep. Troelsen (1965) observed that the use of the Cr_2O_3 paper pellet resulted in some variation in the concentration of Cr_2O_3 in hourly fecal collections. However, digestion coefficients calculated from analyses of fecal samples collected at random for several days agreed closely with those determined with the conventional method.

Factors affecting coefficients of apparent digestibility

As early as 1897 Jordan and Jenter observed that dry matter digestibility by sheep decreased by approximately 5.0 percentage units when the plane of nutrition was increased. Coefficients of digestibility of organic matter, protein and nitrogen-free extract declined at similar rates. Digestibility studies conducted by Eckles (1911, 1913) showed that digestion coefficients determined at maintenance levels were not applicable to cows in heavy milk production. Data supporting this conclusion were reported by Blaxter (1961), Blaxter and Wainman (1961, 1964) and Mitchell and Hamilton (1932, 1941).

Moe et al. (1963) reported TDN values of rations composed of concentrates and either early or late cut hay at several levels of intake as percentages of the TDN determined at maintenance. A total of more than 60 observations were taken with cows producing from 0 to 120 lb of milk per day; as the intake level increased from maintenance to six times maintenance, the relative TDN values decreased from 100 to 77%. In other words a ration which had a TDN value of 82% at maintenance would have a TDN value of approximately 63% at six times maintenance.

It has also been recognized that changes in the proportions of ingredients in the ration may affect the apparent digestion coefficients. Mumford et al. (1914) reported higher digestion coefficients of dry matter, carbohydrate, protein and fat when the ratio of clover hay to ground corn was decreased from 1:1 to 1:5 by weight. Replacing one part of ground corn with linseed meal moderately improved digestibility. Similar results were obtained by Bloom et al.(1957a) who conducted digestibility studies with lactating cows using four ratios of hay to grain. From the high- to low-hay rations, dry matter digestibility increased from 54.5 to 63.1%.

In general, it seems evident that an increase in consumption of a ration, which does not change in composition, apparently decreases digestibility (Moe et al., 1963; Reid, 1961). However, in practice increased nutrient allowances usually are in the form of a greater proportion of concentrates,which should increase digestibility. Thus the overall effect on ration utilization will depend on the relative importance of each of these factors. Moe et al. (1965) observed that when dairy cows were fed rations containing 48 to 75% of concentrates, a depression in the TDN value of 4.0% was found per increment of intake equivalent to the maintenance requirement for TDN. Brown et al. (1964) noted a definite depression in apparent

digestibility with increasing levels of intake of rations containing either 66 or 80% of grain.

Quality of the roughage also has an effect on digestibility since a greater decrease in digestibility will be obtained with increased intake of a mixed ration containing poor-quality hay than with one containing high-quality hay. As a result, Van Soest and Marcus (1964) and Waite et al. (1964) postulated that if forage quality is a factor in determining the depression in digestibility, the cell-wall constituents, as well as the per cent of TDN as cell-wall constituents, may be important items to consider.

Ration effects on fermentation in the rumen

Tappeiner in 1883 (cited by Annison and Lewis, 1959) demonstrated that the fermentation of cellulose in the rumen of the ox resulted in the formation of large amounts of volatile fatty acids (VFA). Elsden (1946) provided the first accurate analysis of the VFA in rumen contents. Until that time, little was known about the production, absorption or metabolism of the individual acids, but it has been established that the volatile acids which are produced in the digestive tract of the ruminant represent an important source of energy to the host (Annison, et al., 1957; Bensadoun et al. 1962; Carroll and Hungate, 1954; Emery et al., 1956).

The amounts and proportions of the VFA produced in the rumen are variable, depending on the nature of the diet, plane of nutrition, time after feeding and age of the animal (Baldwin, 1962; Barnett and Reid, 1961; Rook, 1961; Stewart et al., 1958). Beitz and Davis (1964) noted that feeding a high grain diet to lactating dairy cows caused a significant decrease in the molar percentages of acetate and butyrate in the rumen liquor, and a significant increase in the molar percentages of propionate and valerate as compared with animals fed a high roughage ration. Several other reports in the literature

have confirmed similar effects of ration on the proportions of acetate, propionate and valerate, but the effects on the proportion of butyrate in the rumen fluid have been variable. Jorgensen et al. (1965) did not obtain a significant change in the proportion of butyrate in rumen fluid after changing from a 50:50 roughage to concentrate ration to an all-pelleted corn ration; Coppock et al. (1964b) and Hinders and Owen (1963) observed a significant increase in butyrate when feeding a high concentrate ration.

Balch and Rowland (1957) and Emery et al. (1956) noted a trend toward a decrease in concentration of total VFA with increasing levels of concentrate. Emery et al. (1958) and Jorgensen et al. (1965) reported that the total rumen fluid decreased as the amount of concentrate in the ration increased. A decrease in total rumen fluid coupled with a decrease in VFA concentration as the amount of concentrate in the ration increased, would result in much lower quantities of ruminal VFA with high-concentrate rations as compared to low-concentrate rations. This suggests a decrease in ruminal digestion and VFA production, but it is recognized that ruminal VFA concentration is only an estimate of VFA production.

The pH of rumen contents has also been noted to decrease when rations high in concentrates as compared to rations high in roughage were fed. Davis et al. (1964) postulated that reduced production of saliva lowered the buffering capacity of the rumen and resulted in a lower pH when large amounts of grain were fed. The lower pH favored microflora which produced propionic acid. Hibbs et al. (1956) also demonstrated that rumen pH was inversely proportional to VFA concentrations, and Sutton et al. (1963) found that absorption of VFA increased as pH declined.

Effects of ration composition and energy intake on milk production and milk composition

Experiments by Castle et al. (1959), Holmes et al. (1957) and Reid and Holmes (1956) demonstrated small increases in milk yield when higher levels of concentrate were fed during the declining phase of lactation. The results of the trials, however, applied only to one part of the lactation and, as Broster (1958) has stated, milk production must be studied in terms of the lactation as a whole and not solely as an instantaneous input/output relationship. Furthermore, it is known that responses in milk yield to given amounts of additional feed vary with the stage of lactation (Blaxter, 1959).

Castle and Watson (1961) indicated that feeding concentrates at 4.1 lb per 10 lb of milk, as compared to 2.2 lb per 10 lb of milk, during the first 84 days of lactation increased milk production. Most of the increased production was accounted for by the greater energy content of the diet or by the simple fact that more total energy was fed. In support of this concept, Elliot and Loosli (1959) fed diets in which the level of estimated net energy (ENE) above the maintenance requirement was held constant. There was no difference in production of fat-corrected milk on diets containing 40, 60 or 80% of the ENE in the form of concentrates. A number of relatively recent studies have indicated that feeding of high concentrate rations resulted in increased milk production (Brown et al., 1962b; Charron, 1962; Murdock and Hodgson, 1962; Olson and Benson, 1962), whereas other reports have indicated no advantage in this respect (Hooven and Plowman, 1963; Rumery and Plum, 1963; Wing and Wilcox, 1963). It was pointed out that type and amount of concentrate and/or roughage fed would be of considerable importance and that cows of high potential for production (Bloom et al., 1957b) would usually respond

to a favourable change in ration composition and consumption.

A problem frequently encountered, as a result of feeding high levels of concentrate, is the depression in the percentage of fat in the milk produced (Beitz and Davis, 1964; Powell, 1941; Tyznik and Allen, 1951). For example, Beitz and Davis (1964) noted that the percentage of milk fat decreased from 3.21 to 1.77 when cows were fed ad libitum roughage plus 24.6 lb of concentrates per day as compared with 5 lb of roughage plus ad libitum concentrates, respectively. Many explanations have been set forth as to the cause for low-fat milk when rations of this type are fed to lactating cows. Several workers have demonstrated clearly that changes in the proportions of volatile fatty acids produced in the rumen are correlated with a decrease in fat test, especially when high grain rations are fed. The most widely supported theory, suggested by Tyznik (1951) and advocated by Balch et al. (1955), Brown et al. (1962a), Jorgensen et al. (1965), Rook (1961) and Storry and Rook (1966), is that the milk fat depression is caused by a decrease in the molar proportion of rumen acetate. It has also been reported that the feeding of acetate salts or acetic acid to cows with low milk fat resulted in recovery towards production of milk more normal in composition (Rook and Balch, 1961; Tyznik, 1951).

More recently Storry and Rook (1966) found that the intraruminal infusion of acetate in cows on a low-hay diet caused increases and decreases in the molar proportions of acetate and propionate, respectively, in the rumen. An increase in the percentage of milk fat was observed, but this accounted for only one-quarter of the depression associated with the low-hay diet. This tends to support the observations by Van Soest and Allen (1959), who found no conclusive evidence for an acetate deficiency when milk fat was depressed. They observed that blood levels of acetate were not

significantly decreased and concluded that the decrease in the molar proportion of acetate in the rumen could be the result of increased production of propionate. It has also been reported that the feeding of sodium propionate tends to cause low milk fat (Hawkins, 1959; Rook and Balch, 1961; Schultz, 1954).

The fact that propionate has a marked antiketogenic activity (Ensor et al., 1959; Felts et al., 1958; Forbes et al., 1941; Lindsay, 1959; Reid and Mills, 1961; Seto et al., 1955) has given rise to another theory of milk fat depression, which suggests that this phenomenon results from a deficiency of beta-hydroxy butyric acid (BHBA) in the mammary gland. Arteriovenous studies (Barry, 1964; Hartmann and Lascelles, 1964) have shown that BHBA is removed from blood in the mammary gland, and the incorporation of labelled BHBA into C_4-C_{10} acids has been demonstrated in tissue preparations of the goat mammary gland (Kumar et al., 1965). By contrast Storry and Rook (1965) found no consistent response in milk fat yield and milk fat composition, especially in the content of C_4-C_{10} acids, as a result of infusions of BHBA in cows receiving a normal diet, or in those receiving a low roughage diet and in which the concentration of BHBA in the plasma had been depressed.

Composition of the ration has also been found to alter the solids-not-fat (SNF) and protein content of milk. Rowland (1946) reported that the SNF content of milk increased from 8.34 to 8.68% when the energy supplied by the ration increased from 75 to 100% of Woodman's standards. It is now widely recognized that restriction of energy to less than the recommended standard causes a marked reduction in milk SNF (Burt, 1957; Elliot, 1962; Rook, 1959; Rook and Storry, 1964). Smaller increases have been obtained by raising the energy intake above the standard (Bishop et al., 1963; Boyd and Mathew, 1962; Castle et al., 1963; Holmes et al., 1960; Huber and

Boman, 1966; Olson, 1965; Rook and Line, 1961). For example, Holmes et al. (1957) fed concentrate at 0, 0.9, 1.8 and 2.7 kg/gal of milk, and noted an increase in SNF from 8.3% on the lowest level of concentrate to 8.6% on the highest level.

Increases noted in milk SNF at high energy intakes have generally occurred in the protein fraction (Boman, 1965; Hotchkiss et al., 1960); however, increases in milk lactose have also been noted in some studies (Huber and Boman, 1966; Rowland, 1946). Rook and Line (1961) fed rations which provided starch equivalent at the level of Woodman's standard, 1.1 kg below and 2.3 kg above the standard. Protein content of milk from cows fed the respective rations averaged 3.19, 3.08 and 3.30%.

A change in the roughage:concentrate ratio of the ration may also affect milk SNF and milk protein. Hotchkiss et al. (1960) fed rations with four roughage:concentrate ratios and found that the protein content of the milk increased significantly when the net energy from concentrate increased from 25 and 45% of the ration to 68 and 85% of the ration. In a study by Huber et al. (1964) with cows on pasture and fed corn ad libitum, increases from 8.92 to 9.16% SNF and 3.31 to 3.45% total protein were noted when compared to cows on pasture with no supplemental feed. Closely related to these effects of energy concentration is the observation (Boman, 1965) that when grains of relatively high fiber content were fed to increase the plane of nutrition of a ration, levels of milk SNF and protein were often diminished. He suggested that a minimum level of fiber in the ration might be needed for maximum production of milk protein. It appears that most of the increase in SNF may be in the protein fraction (Hand, 1955; Moody et al., 1964); and in fact, in several instances (Boman, 1965; Burt, 1957) there has been a significant increase in per cent protein but not in total SNF.

Changes in per cent protein and in per cent SNF may be caused by alterations in volatile fatty acids produced in the rumen. Rook (1959) and Rook and Storry (1964) suggested that the higher concentration of propionate produced in the rumen on high grain rations and carried through the portal circulation to the liver, might affect degradation and synthesis of amino acids at this site. Rook and Line (1961) noted higher levels of α-amino nitrogen in blood plasma of cows fed high grain rations as compared to those in blood plasma of cows fed normal amounts of grain. Therefore, increases in milk protein might be the result of higher concentrations of blood amino acids available to the mammary gland for protein synthesis.

Effects of ration and stage of lactation on the fatty acid composition of milk fat

Milk fat synthesis in the ruminant may be viewed as consisting of two stages: one involving the supply of fatty acids, and the other the incorporation of these acids into milk lipids. There are two sources affecting the supply of fatty acids; namely, fatty acids absorbed from blood lipids into the mammary gland (Luick and Lucas, 1962) and fatty acids synthesized from acetate and BHBA in the mammary tissue (Kumar et al., 1959; Popjak, et al., 1951). Several reports (Luick, 1961; Luick and Lucas, 1962; McCarthy and Patton, 1963; Patton and McCarthy, 1963; Patton et al. 1962) have established that the second stage is accomplished largely, if not completely, within the mammary gland.

With respect to the fatty acid pool created from blood lipids, McCarthy et al. (1960) suggested, on the basis of triglyceride structure, that the blood triglycerides must be rearranged when they are synthesized into milk fat. The observation by Lauryssens et al. (1961), that stearate in the blood perfusing the isolated bovine udder is converted in part to

oleate in the mammary tissue, provides an explanation of the differences in oleate:stearate ratios between blood and milk lipids.

Patton and McCarthy (1963) postulated the existence of a fatty acid pool in the mammary gland, in which blood fatty acids predominated (long chain fatty acids), and one in which cell-synthesized fatty acids predominated (short chain fatty acids). Each of these pools contributed to the triglyceride formation. Diglycerides, consisting mainly of long chain fatty acids, were synthesized first and the triglyceride was then synthesized by incorporation of a short chain fatty acid.

The principal fatty acids measured in milk fat are the 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 16:1, 18:0, 18:1, 18:2 and 18:3 fractions. Jensen et al. (1962) obtained average values for the major fatty acid fractions over a one-year period and recorded the percentages as follows: 4:0 - 3.75, 6.0 - 2.22, 8:0 - 1.17, 10:0 - 2.54, 12:0 - 2.81, 14:0 - 10.06, 16:0 - 24.97, 18:0 - 12.07, 18:1 - 27.09, 18:2 - 2.39 and 18:3 - 2.06. However, these workers stated that seasonal trends were noticeable, with 18:0 and 18:1 higher in the summer than in other seasons. This trend was reversed with 12:0, 14:0 and 16:0 fatty acids. A more thorough investigation concerning lactational changes in fatty acid composition of milk fat was carried out by Stull et al. (1966). These workers noted that the 6:0, 8:0, 10:0, 16:1 and 18:2 fractions increased as the lactation progressed, whereas a reverse pattern was noted for the 16:0, 18:0 and 18:1 acids. The 12:0 and 14:0 fatty acids showed an increase during the first part of the lactation followed by a decrease later in the lactation. Per cent 18:3 appeared to be cyclic in nature, with its highest value around the 40th week of lactation.

The amounts and proportions of these acids depend on the availability of each individual fatty acid in the mammary tissue at the time of the

formation of a triglyceride. Beitz and Davis (1964) and Jorgensen et al. (1965) observed that the feeding of rations high in grain and low in roughage caused a significant decrease in the saturated fatty acids, with a corresponding increase in the unsaturated analogs. The greatest change was found in stearic acid. Dronawat et al. (1966) noted that when animals were fed a ration which depressed the percentage of milk fat, proportionally more 14:0, 18:1 and 18:2 and less 16:0 and 18:0 fatty acids were found in milk fat.

A ration which does not supply sufficient energy for maintenance and production of the cow during early lactation will result in loss of body weight because of catabolism of the body fat stores. This mobilization of body fat supplies a large amount of long chain fatty acids which undoubtedly could affect the fatty acid composition of milk fat. Stull et al. (1966) hypothesized that at the beginning of the lactation there was an initial surge of 16:0, 18:0 and 18:1 acids (the main components of tallow) into the blood stream and hence into the udder. This mobilization of body fat would theoretically decrease during the lactation as body weight decreased at a decreasing rate and have less effect on the composition of milk fat.

Ration effects on energy utilization for milk production

The energy standards for milk production used until recently (NAS-NRC, 1958; Morrison, 1956) were based primarily on the early work of Haecker (1914) in which the average milk production was less than 25 lb per day. Considerable data have been published in recent years indicating that cows may not reach their genetic potential for milk production when fed according to these standards (Brown et al., 1962b; Huffman, 1961; Kesler and Spahr, 1964). Thus cows at high levels of milk production may require

more gross energy per unit of milk than cows at low levels of production. Reid (1956, 1961) suggested that the major reason for increased gross energy requirements per unit of milk by high producing cows was a decreased digestibility of the diet when fed in sufficient amounts to support high milk production. NAS-NRC Nutrient Requirements of Dairy Cattle (1966) was an attempt to provide energy requirements which would be adequate for cows at all levels of milk production.

Efficiency of milk production is generally expressed as the amount of estimated net energy (ENE), digestible energy or total digestible nutrients (TDN) required per lb of FCM produced. Hinders and Owen (1963) found that the ENE consumed (exclusive of maintenance requirements) per lb. of FCM averaged 0.35 mcal for all rations and did not differ significantly with changes in the roughage to concentrate ratio. This was in agreement with previous reports indicating that ENE gave a more accurate evaluation of rations varying in roughage to concentrate ratio than did digestible energy or TDN (Elliot and Loosli, 1959; Irvin et al., 1951; Loosli et al., 1955; Saarinen et al. 1951).

Hinders and Owen (1963) noted a significant decrease in the requirement of digestible energy and TDN per lb of FCM with increasing proportions of concentrates in the ration. This agreed with the reports of Huffman and Duncan (1949), Irvin et al. (1951) and Smith et al. (1945). Jumah et al. (1965) found that the average requirement above maintenance for the production of a lb of FCM was 0.579 mcal digestible energy or 0.287 lb TDN. It is interesting to note that Armsby in 1917 reported a similar requirement per lb of FCM. Jumah et al. (1965) noted that the requirements per lb of FCM produced from the highest to the lowest levels of milk production ranged from 0.245 to 0.333 lb TDN. This could have been the result of a decrease in the proportion of concentrates in the ration, since the roughage:

concentrate ratio increased from 0.32 to 0.41 from the first to the eighth month of lactation.

The nature of the effect of ration composition on TDN requirements for milk production is not fully understood. Hinders and Owen (1963) speculated that a decrease in the concentration of VFA and a reduction in the ratio of acetate to butyrate contributed to the more efficient use of energy when hay was replaced by increments of concentrate in the ration.

To express the digestible energy intake per lb of FCM on a comparable basis with the actual energy produced as milk, gross efficiencies are sometimes calculated (Brody, 1945). Gross efficiency is obtained by dividing the milk calories produced by the digestible feed calories consumed. Putnam and Loosli (1959) fed animals ad libitum but maintained roughage:grain ratios at 80:20, 60:40 and 40:60. Although milk production showed slight increases in response to higher proportions of concentrates, little difference was noted in the gross efficiency. Flatt et al. (1966) fed cows ad libitum with rations containing roughage:grain ratios of 60:40, 40:60 and 20:80 and observed that metabolizable energy consumed remained approximately equal with all rations. Milk fat test was depressed, and milk energy produced actually declined with the increasing energy concentration; this caused the gross efficiency to decline from 33.8 to 32.3 to 26.0% with the respective ratios. These results of Flatt et al. (1966) suggest that high-grain feeding is not always efficient. A factor which could have had a marked effect on the above observation was the change in body weight for the three groups fed different roughage to concentrate ratios. In fact Van Soest (1963) postulated that a fundamental antagonism exists between metabolism for milk production and that for rapid gains in body weight. Thus a ration which contains a proportion of concentrate high enough to cause milk fat depression is not as efficient for

milk production as one containing less grain but which still meets nutrient requirements.

To consider the energy necessary for maintenance and the energy lost or gained by a change in body weight, net energetic efficiencies, which take these variables into account, can also be calculated (Brody, 1945). However, corrections for maintenance and body weight change involve some critical assumptions regarding the caloric changes applicable to a specific cow. For example, Flatt (1966) observed that it was not unusual for cows to lose 100 to 200 lb of body weight during the first 75 days after calving; and some cows have been noted to lose 400 lb. The caloric value of these weight losses is difficult to assess and the negative energy balances could be considerably greater than those which have been studied by energy metabolism workers in the past (Bath et al., 1965; Flatt et al., 1965). Furthermore, Wallace (1959) suggested that the maintenance requirement increases throughout lactation. Although it appears that errors might occur in the estimates of energy necessary for maintenance and the energy supplied or lost as body weight, net energetic efficiency is an attempt to take these variables into consideration when estimating energy utilization for milk production.

Jumah et al. (1965) obtained net energy values throughout the lactation of high-producing dairy cows, and noted that net energetic efficiency for milk production decreased from 70% during the first month to 51% during the eighth month of lactation. They postulated that the higher net energetic efficiency values during early lactation were the result of an underestimation of the energy supplied by the metabolism of body fat. Concomitant losses of body fat and gains in body water would tend to keep body weight constant and mask the energy contribution made by the body stores. A second factor which could have contributed to the apparent high efficiency during

early lactation might have been the higher proportion of concentrate in the ration at early stages of lactation.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

General outline

The experimental work reported in this thesis is divided into two parts. Experiment 1 involved a study of the use of chromic oxide as an indicator in the determination of apparent digestion coefficients. In Experiment 2 a comparison was made of the effects of rations, composed of different levels of roughage and concentrate and fed at various planes of energy nutrition, on the performance of lactating dairy cows.

Experiment 1

Concentrations of chromic oxide, crude protein, phosphorus and calcium in the feces of the lactating dairy cow

Objectives

The main objective of the experiment was to study the use of chromic oxide (Cr_2O_3) as an indicator of fecal excretion in digestion studies with lactating dairy cows. Since it has been shown that the fecal concentration of Cr_2O_3 changes with time during a 24-hour period when Cr_2O_3 is added to sheep and steer rations (McGuire et al., 1966; Troelsen, 1965), it was thought that other fecal constituents might follow a similar trend. Consequently, additional analyses were carried out to determine the concentrations of crude protein, phosphorus and calcium in the excreta at different times during the day.

Experimental

Animals and treatments

Four Holstein-Friesian cows from the University dairy herd were allotted as A, B, C and D. These cows were fed to maximum appetite, and their rations had roughage to concentrate ratios of 1.7:1,1:1, 1.4:1 and 1:2:3, respectively. The roughage fed in this study consisted of a field cured alfalfa-brome grass hay rated as good to excellent in quality. The formulation of the concentrate fed is outlined in Table 1.

Animals were fed concentrate at 530 and 1530 hours and roughage at 700 and 1700 hours.

Because the cows calved at different dates, tests were conducted with cows A and B eight weeks prior to those with cows C and D. All tests were carried out over a period of eight days, and were conducted at the approximate peak of feed intake at 5 to 6 weeks after each cow calved.

Table 1. Formulation of the concentrate ration

Ingredients	%
Barley	56.0
Oats	25.0
Soybean oil meal	10.5
Bran	3.0
Molasses	2.0
Co-I salt	1.0
Trisodium phosphate	2.0
Vitamin premix[1]	0.5
	100.0

[1]Vitamin premix: Soybean oil meal, 3.6 kg; vit. A (10,000 I.U./g), 360 g; vit. D_2 (35,000 I.U./g), 22 g.

Administration of Cr_2O_3

Cr_2O_3 absorbed onto paper,[1] was compressed into 10 g pellets (Troelsen, 1963). The level of Cr_2O_3 in the paper was 34.60% on a dry matter basis. Pellets were administered with a balling gun daily at 900 hours for the eight day period. Cows A and B were given one pellet daily, or 3.46 g of Cr_2O_3. This gave very low levels of fecal Cr_2O_3, and it was found that chemical methods for Cr_2O_3 determination were of limited precision at this low concentration. Therefore, three pellets, or 10.38 g of Cr_2O_3 , were administered daily to each of cows C and D.

The first three days of the test were used as a preliminary period to enable the concentration of Cr_2O_3 in the feces to reach a 'steady state', which could be expressed as being the maximum recovery from the feces of the Cr_2O_3 administered to the cow. The remaining five days were used for

[1]Rowett Research Inst., Bucksburn, Scotland, Great Britain.

collections of total feces.

Collection and sampling of feces

The apparatus for collection of feces, separated from urine, was similar to that described by Balch et al. (1950), except that harnesses were constructed of webbing straps cut to fit each cow and the fecal bags were 19" wide and 29" deep and contained an inner plastic liner.

Fecal collections commenced at 2100 hours of the third day of the trial and lasted for five full days. The inner plastic bag was removed and replaced by an empty bag at four-hour intervals. The feces were weighed immediately and the total weight of the six individual daily collections was recorded as total daily excreta per cow.

A 5% aliquot was taken from each of the four-hour collections, dried in a forced draft oven[2] at 40°C, ground in a laboratory mill[3] and stored for further analysis. In addition, a 2% aliquot was taken at each collection, and at the end of 24 hours, the six samples were composited to provide a representative sample for the day. These composite samples were treated as above and stored for further analysis.

Chemical analysis

Dry matter and nitrogen were determined on fecal samples by AOAC (1960) methods. Determination of Cr_2O_3 in the Cr_2O_3 paper and fecal samples was according to the method of Christian and Coup (1954). Calcium was measured by the method of Patton and Reeder (1956) as modified by Findlay and Delong (1957). Phosphorus was determined according to the method described by Dickman and Bray (1940) as modified by Woods and Mellon (1941).

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[2]Despatch Oven, Co., Minneapolis, U.S.A.

[3]Arthur H. Thomas Co., Philadelphia, Pennsylvania, U.S.A.

Statistical analysis

Means, standard deviations and correlations were calculated (using a Cogito 566 PR calculator[4]) according to the methods outlined by Steel and Torrie (1960).

Results and Discussion

The daily mean percentage recovery of Cr_2O_3 in the feces, digestion coefficients for dry matter determined by the conventional and Cr_2O_3 methods and the standard deviations are listed for each animal in Table 2.

Recovery of Cr_2O_3

The percentage recovery of Cr_2O_3 varied with different animals. Average percentage recovery in feces from animals B, C and D was in relatively close agreement, with averages slightly above and below 100 per cent. Average recovery in feces from animal A was only 84%. The lack of agreement in average recovery of Cr_2O_3 from animals A and B, both of which were given 3.46 g of Cr_2O_3 daily, might have been attributable to the low concentrations of Cr_2O_3 in the feces from these cows, which resulted in much lower precision in the chemical analysis (difficulties in determining the end point of the titration). Higher concentrations of Cr_2O_3 in feces from animals C and D, which were given 10.38 g of Cr_2O_3 daily, enabled greater precision in the chemical analysis; close agreement was obtained in average recovery of Cr_2O_3 in the feces from these cows during the five day collection period.

The recovery of Cr_2O_3 excreted by animals C and D (92.3 and 95.2% of that administered) was in good agreement with the results of other experiments using the Cr_2O_3 paper method (Cowlishaw and Alder, 1963; Langlands et al., 1963a; Troelsen, 1965). However, Corbett et al. (1960) observed about 100% recovery when the Cr_2O_3 was administered as Cr_2O_3 paper.

[4]SCM Marchant, Division of SCM Corporation, Diehl Rechenmaschinenfabrik, Nürnberg, West Germany.

Table 2. Percentage recovery of Cr_2O_3 in the feces and coefficients of apparent digestibility of dry matter as determined by conventional and Cr_2O_3 methods

Animal	Cr_2O_3 recovery %	Apparent dry matter digestion, conventional method %	Apparent dry matter digestion, Cr_2O_3 method %
A	84.5 ± 13.0[a]	67.7 ± 3.5[a]	57.5 ± 2.6[a]
B	103.6 ± 15.8	65.9 ± 3.9	62.7 ± 8.9
C	95.2 ± 6.2	67.9 ± 4.9	65.1 ± 5.1
D	92.26 ± 16.2	64.6 ± 10.5	59.2 ± 13.6

[a]Standard deviation

To show the magnitude of variation during the day in Cr_2O_3 excretion, the concentration of Cr_2O_3 in the feces at the six sampling intervals was expressed as a percentage of the daily mean concentration, and average values calculated for the five day period. The diurnal excretion patterns of Cr_2O_3 in the four-hour fecal collections (Fig. 1) show substantial variation at any given time. Variation was more pronounced when less Cr_2O_3 was administered (cows A and B versus C and D). The recovery of Cr_2O_3 during the day ranged from 76.5 to 123.0% $\pm$ 20.7 at 100 and 1300 hours, respectively, with animals A and B, whereas a range of 86.8 to 113.9% $\pm$ 12.0 was obtained at 100 and 900 hours, respectively, with animals C and D.

These variations are in agreement with the reports of McGuire et al. (1966), Haenlein et al. (1966) and Troelsen (1965), who noted that the time during the day for optimum, maximum and minimum recovery of Cr_2O_3 varied extensively.

Dry matter digestibility

Each coefficient of dry matter digestion obtained by the conventional

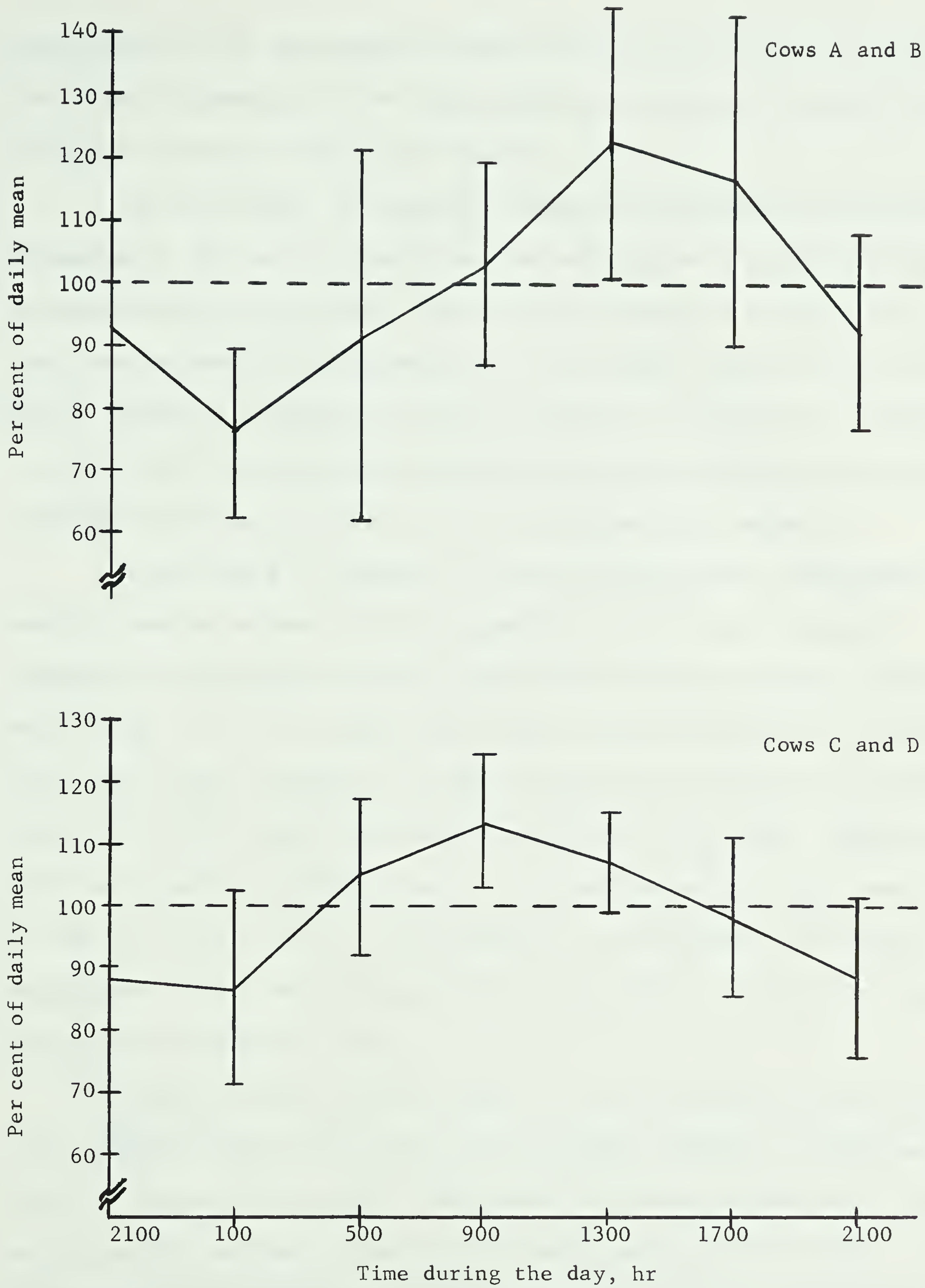

Fig. 1. Diurnal excretion patterns of Cr_2O_3, expressed as a percentage of the daily mean obtained with the conventional method. The vertical lines represent the standard deviations.

method (Table 2) is an average of results from the five composite samples. Coefficients determined by the Cr_2O_3 method are averages of results from the 30 samples obtained at four-hour intervals.

On the average, the apparent digestion coefficients for dry matter determined by the total collection method were higher ($P < .01$) than those obtained with the Cr_2O_3 method. However, with animals B,C and D, the conventional method resulted in values only 4 to 9% higher than with the Cr_2O_3 method, whereas a difference of 18% was obtained with animal A. Consequently, it appears that the significant difference in results between the two methods could be attributed primarily to the data obtained with animal A.

Coefficients of apparent dry matter digestibility, determined with the Cr_2O_3 method at each of the six sampling intervals per day, were expressed as percentages of the coefficients determined with the conventional method (Fig. 2). It is evident from these illustrations that the two methods resulted in closer agreement in average digestibility during the day with cows C and D (85 to 102%) than with cows A and B (75 to 102%). Moreover, there was greater variation at most of the sampling periods with cows A and B, than with cows C and D. This emphasizes the superiority of estimates of coefficients of dry matter digestibility when 10.38 g of Cr_2O_3 was administered daily instead of 3.46 g.

From the results shown in Fig. 2, it can be observed that fairly close agreement between the Cr_2O_3 and conventional methods was obtained at 500, 900, 1300 and 1700 hours. Using data from these periods only, it was calculated that estimated digestibility would average 94.2% of that obtained with the conventional method for animals A and B, and 97.5% for animals C and D. It was concluded that fecal grab samples obtained over a period of five days at the hours noted above, after administering 10.38 g

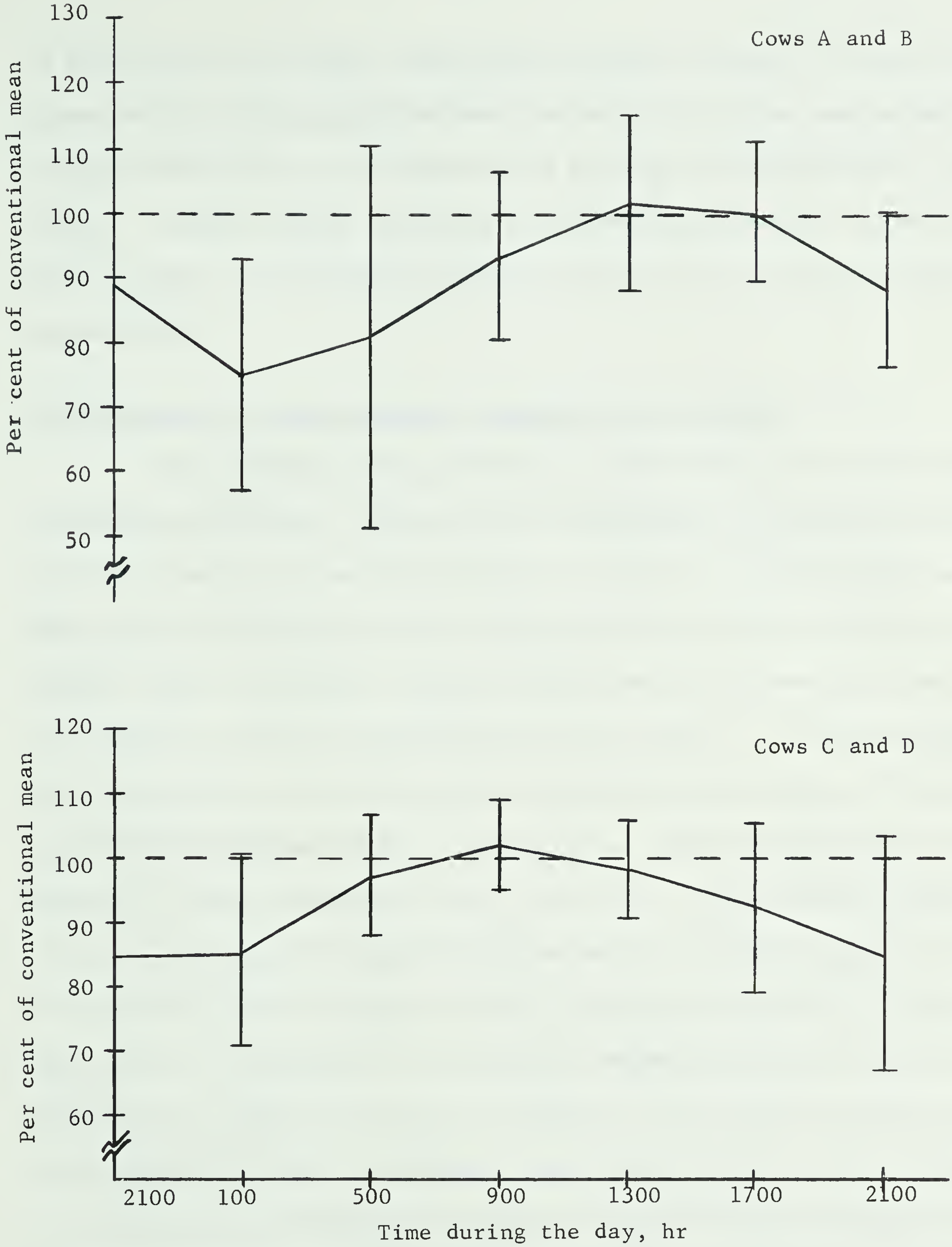

Fig. 2. Dry matter digestion coefficients, determined by the Cr_2O_3 method at different times during the day, expressed as a percentage of the daily mean obtained with the conventional method. The vertical lines represent the standard deviations.

of Cr_2O_3 daily per animal, would give reasonable accuracy in prediction of digestibility in the main experiment, which would involve more animals but similar variation in the proportions of roughage and concentrate in the ration. Troelsen (1965) concluded that several grab samples taken at random over at least two consecutive days were required for accurate estimates of digestibility.

Concentrations of crude protein, phosphorus and calcium

The variations during the day in concentration of crude protein, phosphorus and calcium, expressed as a percentage of the daily mean concentration of each of these ingredients in the feces, are illustrated in Fig.3. There was some variation in the concentrations of these nutrients in fecal samples taken at 500, 900, 1300 and 1700 hours, but it was much less than the variation noted in concentration of Cr_2O_3 (Fig. 1). On the average, the concentrations of crude protein, phosphorus and calcium in these samples were 101.3%,100.6% and 96.7%, respectively, of the concentrations in the composite samples obtained by total collection. Non-significant correlations of 0.27, 0.36 and 0.47 were obtained between the diurnal excretion pattern for Cr_2O_3 and those for crude protein, phosphorus and calcium, respectively. This would be attributable to the smaller variation obtained in fecal concentrations of these nutrients, as compared to the greater variation in concentration of Cr_2O_3 at different times during the day. Since concentrations of crude protein, phosphorus and calcium in feces did not vary appreciably at different sampling intervals, it appeared that fecal grab samples would enable reliable estimates of excretion of these nutrients.

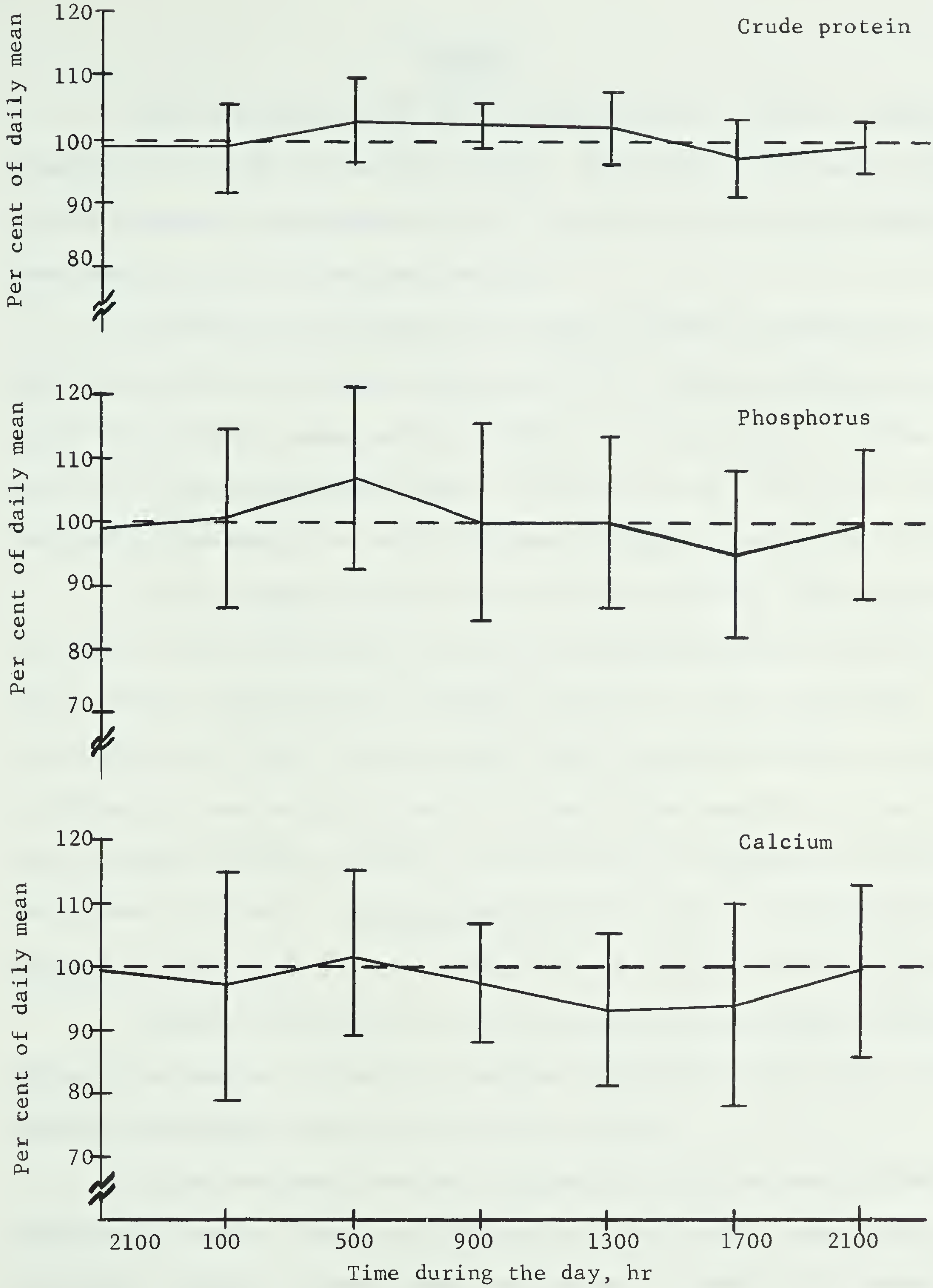

Fig. 3. Diurnal excretion patterns of crude protein, phosphorus and calcium, expressed as a percentage of their daily means obtained with the conventional method, for all experimental animals. The vertical lines represent the standard deviations.

Summary

Cr_2O_3 recovery in the feces varied from 84 to 103% for animals A and B and from 92 to 95% for animals C and D. The apparent dry matter digestion coefficients were significantly lower ($P < .01$) when the Cr_2O_3 method was used instead of the conventional method.

Estimates of the apparent dry matter digestion coefficients, using the Cr_2O_3 method with samples taken at 500, 900, 1300 and 1700 hours, were 94.2% and 97.5% for animal pairs A, B and C, D respectively, of those obtained by the conventional method. This illustrates the better estimate obtained by increasing the daily allotment of Cr_2O_3 from 3.46 to 10.38 g.

On the average, concentrations of crude protein, phosphorus and calcium in fecal grab samples were 101.3, 100.6 and 96.7%, respectively, of their average concentrations in samples obtained by total collection. Since concentrations of these nutrients were fairly uniform in samples collected at different times during the day, it appeared that analysis of grab samples would provide reliable estimates for calculation of digestion coefficients for dietary nutrients. Cr_2O_3 excretion patterns were not significantly correlated with the crude protein, phosphorus and calcium excretion patterns.

Standard deviations for both the Cr_2O_3 and conventional methods of determining apparent dry matter digestion coefficients were high; thus showing considerable variation for either method.

It was concluded that the Cr_2O_3 method could be used for the determination of apparent digestion coefficients under similar experimental conditions. Results agreed closely with those obtained by the conventional method when 10.38 g of Cr_2O_3 was administered at 900 hours daily for an eight day period, and fecal grab samples were taken at 500, 900, 1300 and 1700 hours daily during the last five days.

Experiment 2

Ration effects on performance of lactating dairy cattle

Objectives

Since the roughage:concentrate ratio in the ration and the plane of energy nutrition play important roles in the dairy cow's performance, an experiment was conducted to study these factors. The experimental treatments for this study consisted of feeding three levels of roughage within each of three levels of energy nutrition.

The experiment was designed to study:

a. Ration effects on dry matter intake.

b. Apparent digestibility of dry matter, crude protein and gross energy.

c. Rumen VFA and rumen pH.

d. Milk production and composition of the milk.

e. Fatty acid composition of milk fat.

f. Energy utilization for milk production.

Experimental

Animals and treatments

Twenty-seven Holstein-Friesian cows from the University dairy herd which began lactations between June 12, 1966 and March 5, 1967 were used as experimental animals. Prior to the experiment, they were divided into three groups of nine on the basis of age, and expected level of milk production as follows:

(1) High production - mature cows expected to produce over 5000 kg milk per 305-day lactation.

(2) Medium production - mature cows expected to produce below 5000 kg milk per 305-day lactation.

(3) Heifers - heifers aged 2-2.5 years at first calving.

As the cows calved, one animal from each group was randomly allotted to one of the nine experimental treatments in a 3 x 3 x 3 factorial design with three animals and three levels of roughage superimposed on each of three levels of energy (Table 3).

Table 3. Experimental design

	Level of roughage, kg/100 kg body weight		
	0.75	1.50	2.50
Level of energy, % of NRC requirements (1966)		Number of cows	
90	3	3	3
100	3	3	3
120	3	3	3

Limited storage facilities made it impossible to feed rations to constant composition during the entire experiment. Consequently, dry matter, crude protein and gross energy varied slightly over the experimental period. Although average values only are reported, values determined in each digestion trial were used in computations of feed consumption by individual cows. The roughage fed in this study consisted of a field cured alfalfa-brome grass hay valued as good to excellent in quality. Formulation of the concentrate ration and the mean chemical composition and energy content of the concentrate and roughage are summarized in Table 4.

Table 4. Formulation of the concentrate and composition of ration ingredients

Concentrate	%	Roughage Alfalfa-brome grass hay
Barley	56.0	
Oats	25.0	
Soybean oil meal	10.5	
Bran	3.0	
Molasses	2.0	
Co-I salt	1.0	
Trisodium phosphate	2.0	
Vitamin premix[1]	0.5	
Total	100.0	
Dry matter, %	88.8	90.1
Crude protein, % of dry matter	15.8	14.6
Gross energy, kcal/gm dry matter	4.3	4.3
Digestible energy, kcal/gm[2]	3.1	2.2

[1]Vitamin premix: Soybean oil meal, 3.6 kg; vit. A (10,000 I.U./g), 360 g; vit. D_2 (35,000 I.U./g), 22 g.

[2]Digestible energy of the ration ingredients was a calculated value based on NRC "Nutrient Requirements of Domestic Animals" (1966).

Feeding of the experimental rations commenced three weeks prior to expected calving. Each cow was fed roughage free-choice; the concentrate allowance was increased gradually so that all cows were consuming all they would eat in the last week before calving. After calving, the concentrate and roughage allowances were increased or decreased gradually until all cows were being fed their designated ration according to body weight and level of milk produced. Quantity of roughage fed (Fig. 4) depended only on the weight of the individual cow and its designated treatment. The absolute amount of roughage supplied was adjusted every second week according to the weight of the cow. The calculated amount of digestible energy supplied by the roughage was supplemented with concentrate to provide 90, 100 or 120% of the digestible energy requirements (NAS-NRC, 1966) according to body

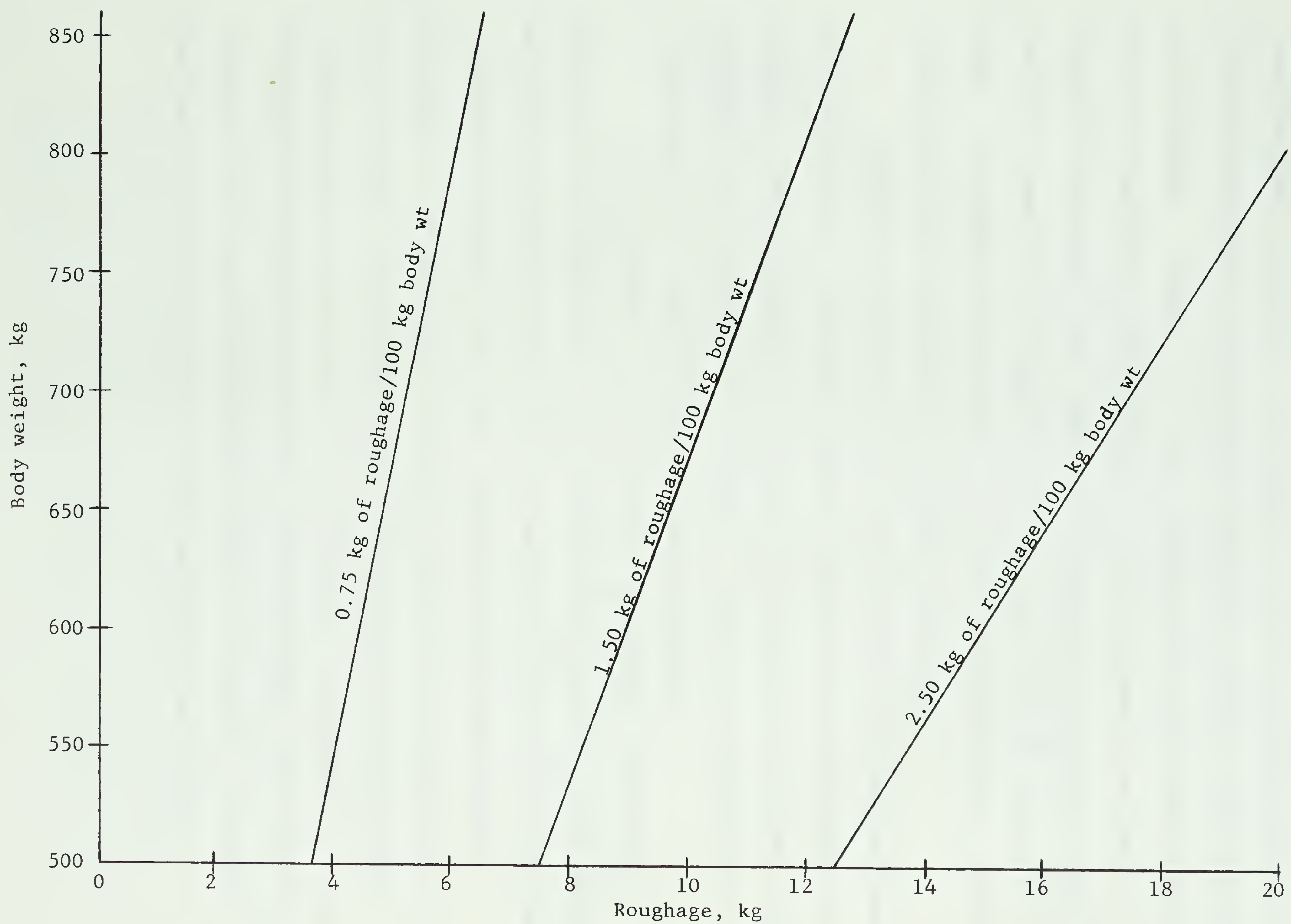

Fig. 4. Daily roughage allowance according to experimental treatment and body weight of the cow.

weight and milk production. The amount of concentrate supplied was adjusted every Wednesday according to the average of the cow's previous seven day solids-corrected milk production (for formula see page 40) and her designated treatment. Figures 5, 6 and 7 illustrate the curves used to calculate the concentrate requirement for the nine experimental rations. To simplify calculations three curves were computed for each ration, from which specific concentrate requirements could be interpolated for cows of varying weight. Throughout the experimental period all cows had access to cobaltized-iodized (Co-I) salt and trisodium phosphate free choice.

Animals were fed concentrate at 530 and 1530 hours and roughage at 700 and 1700 hours. Unconsumed feed was removed before each feeding and weighed. Weekly allotments of concentrate and roughage fed and the rejected ingredients were recorded for each animal. Animals were weighed at the commencement of the experiment and bimonthly thereafter.

The experimental period lasted for 28-44 weeks after calving. In general, in this experiment a cow's lactation was considered to be completed when her milk production decreased to less than 10 kg per day, or she had completed a lactation of 44 weeks. Three cows were removed from the experiment by the 28th week of lactation because of low production; one in each of the 20th, 25th and 28th weeks. Milk production during the last week by the two cows dried off prior to 28 weeks on the experiment was used to complete statistical analysis for the 28-week period. Since increasing numbers of cows were dried off after 28 weeks of lactation, statistical analyses of milk production for a longer period were not possible.

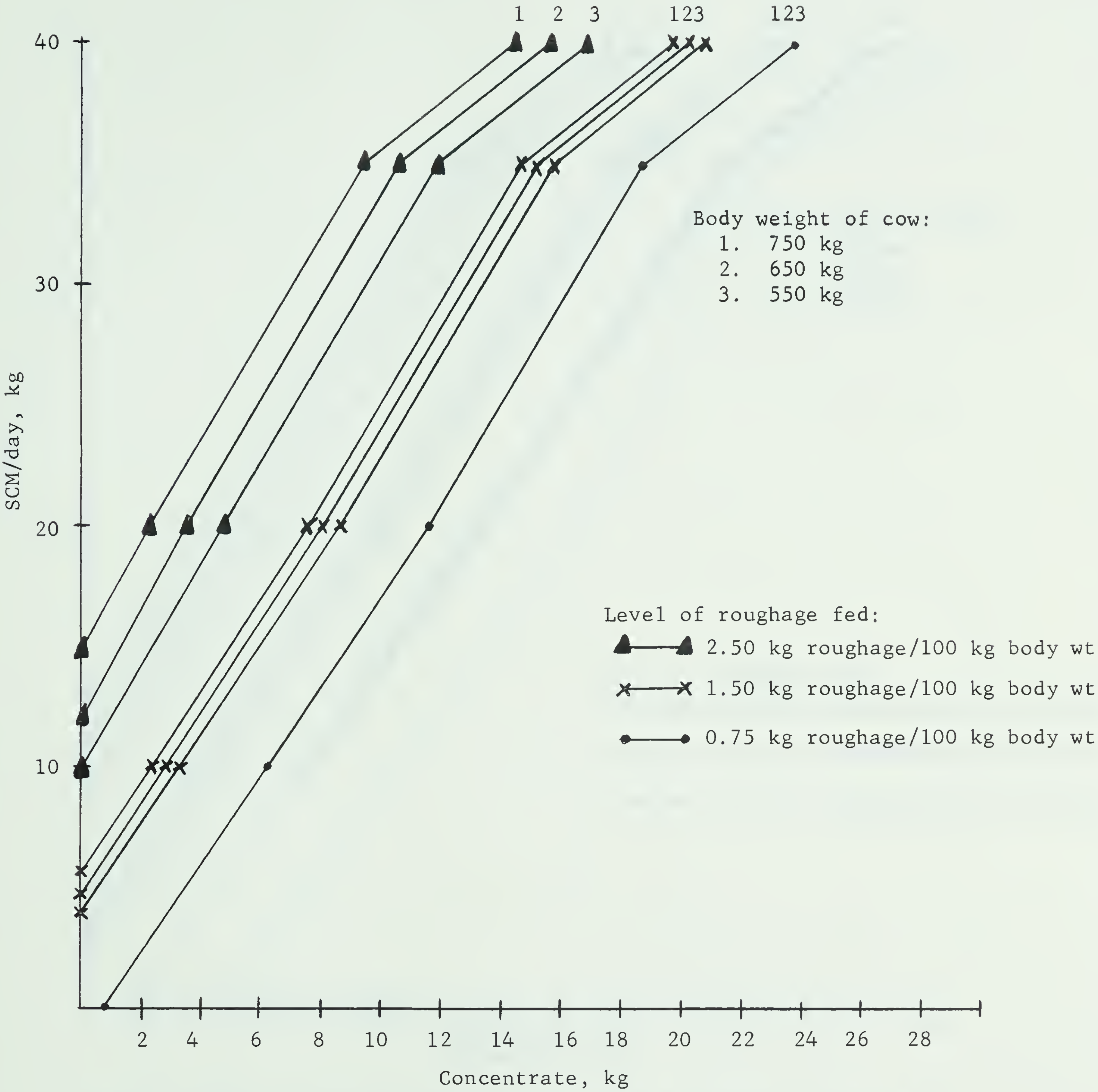

Fig. 5. Daily concentrate regimen for cows fed energy at 90% of NRC requirements; adjusted for SCM production, level of roughage fed and body weight of the cow. (Heifers were fed additional concentrates as follows: 500 kg heifer - 1.7 kg; 550 kg heifer - 1.1 kg. In the last two months of pregnancy additional concentrates were fed as follows: 550 kg cow - 3.8 kg; 650 kg cow - 4.3 kg; 750 kg cow - 4.9 kg).

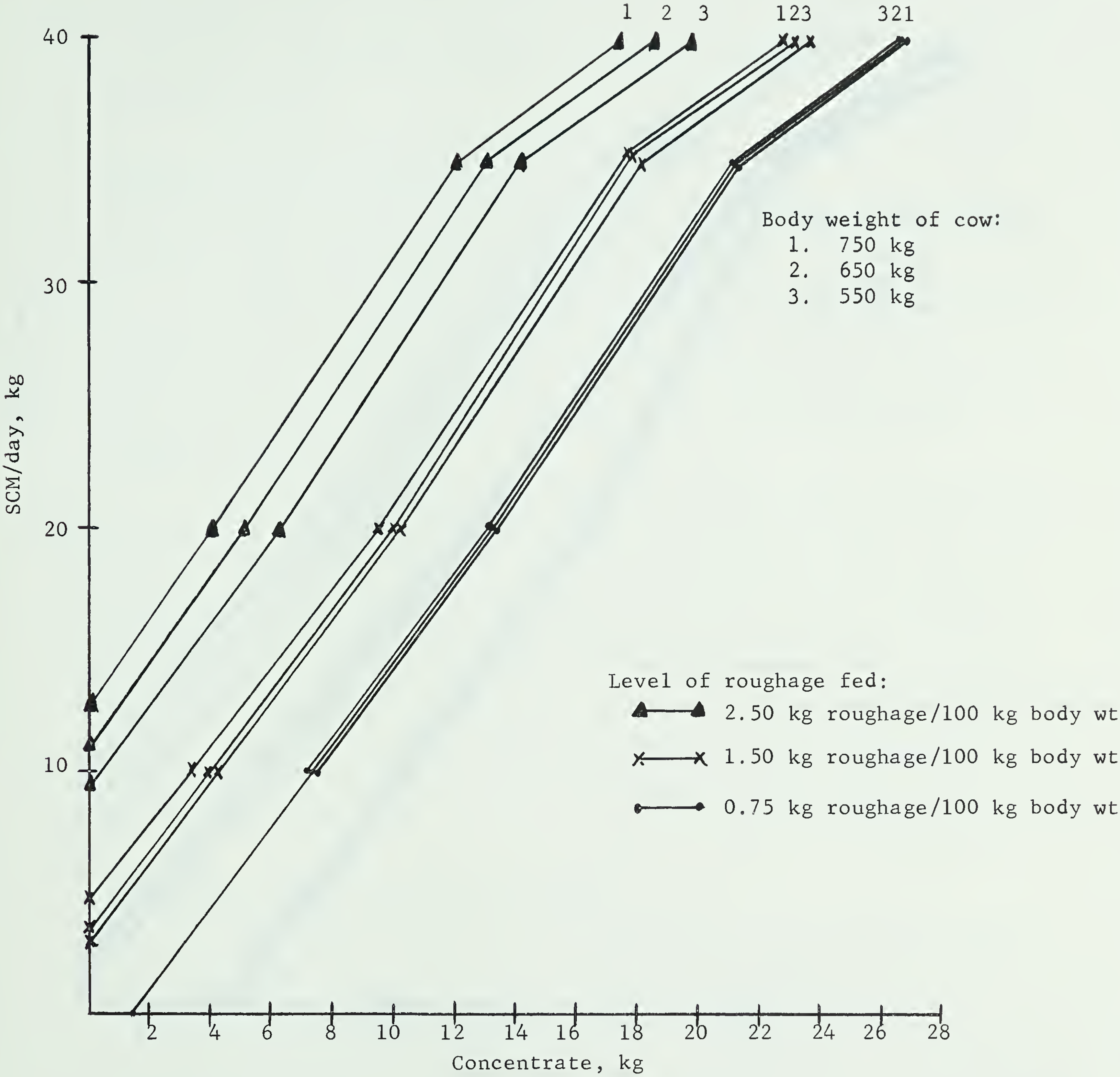

Fig. 6. Daily concentrate regimen for cows fed energy at 100% of NRC requirements; adjusted for SCM production, level of roughage fed and body weight of the cow. (Heifers were fed additional concentrates as follows: 500 kg heifer - 1.9 kg; 550 kg heifer - 1.3 kg. In the last two months of pregnancy additional concentrates were fed as follows: 550 kg cow - 4.3 kg; 650 kg cow - 4.8 kg; 750 kg cow - 5.4 kg).

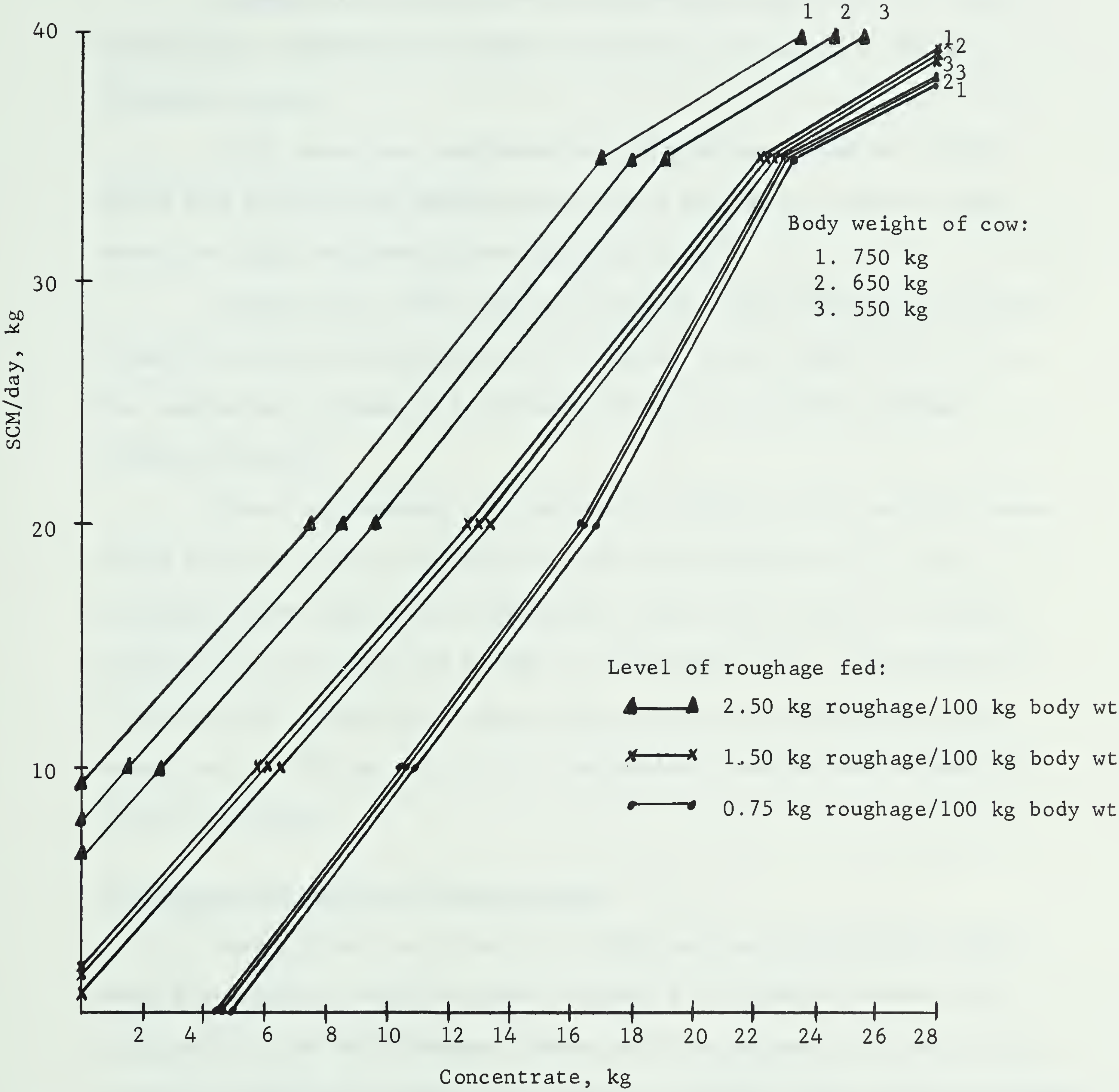

Fig. 7. Daily concentrate regimen for cows fed energy at 120% of NRC requirements; adjusted for SCM production, level of roughage fed and body weight of the cow. (Heifers were fed additional concentrates as follows: 500 kg heifer - 2.3 kg; 550 kg heifer - 1.9 kg. In the last two months of pregnancy, additional concentrates were fed as follows: 550 kg cow - 5.1 kg; 650 kg cow - 5.8 kg; 750 kg cow - 6.5 kg).

Digestion studies

Digestibility studies were conducted when animals were at their maximum milk production, and again just prior to termination of their lactation period.

Cr_2O_3 paper was compressed into 10 g pellets (Troelsen, 1963). Three 10 g pellets were administered, with a balling gun, daily at 900 hours for eight days during each digestion study.

Samples were taken from the concentrate and roughage fed and also from the rejected concentrate and/or roughage of each animal under trial. The samples were ground in a laboratory mill and stored for further chemical analysis.

Fecal grab samples were obtained at 500, 900, 1300 and 1700 hours daily during the 4th to 8th days of each of the digestibility trials. Individual grab samples were immediately frozen until dried in a forced draft oven for 48 hours and ground in a laboratory mill. Ten grams were taken from each ground grab sample and composited to provide one fecal sample per cow during each trial. The composite samples were stored for chemical analysis.

Collection and sampling of rumen liquor

Rumen liquor was obtained, at 900 hours on the first day after each digestibility study had ended, through a 1" diameter stomach tube attached to a set of Erlenmeyer flasks and a vacuum pump. To obtain a more uniform representative sample from each cow, drinking water was not supplied to the animals from 500 hours until time of collection. The collected rumen fluid was filtered through six layers of cheese cloth and pH determined using a Photovolt model 125 electronic pH meter, immediately after collection. Twenty-five ml were acidified to a pH of less than 2 by adding 0.5 ml of

50% (v/v) H_2SO_4 in a 90 ml centrifuge tube. The contents of the tube were thoroughly mixed with a glass rod, and after standing for 30 minutes, centrifuged for twenty minutes at 5000 revolutions per minute. The supernatant was poured into glass vials and stored at 4°C for chemical analysis.

Weighing and sampling of milk

Daily milk production, measured by a Milkoscope[5], was recorded for each cow. During the first three months of lactation milk samples were obtained on the first and third Tuesday of the month, consisting of a constant proportion of the milk from the last two milkings; thereafter, similar milk samples were taken once per month. Tests were conducted on milk samples from each cow to determine percentages of fat, protein and solids-not-fat (SNF). The remainder of each sample was stored at 4°C; and within 24 hours approximately 150 ml was lyophilized in a freeze-drying apparatus.[6] The lyophilized milk samples were then stored at -18°C for further chemical analysis.

To enable uniform comparisons between animals producing milk of varying composition, all data of milk production were converted to fat-corrected milk (FCM) and solids-corrected milk (SCM). FCM was obtained by using the formula (Overman and Gaines, 1948):

FCM (kg) $= 0.4M + 15\ F$; SCM was calculated by the formula (Tyrrel and Reid, 1965): SCM (kg) $= 12.3 + 6.56\ SNF - 0.0752\ M$.

Where: M = milk production in kg

F = butterfat in kg

SNF = solids-not-fat in kg

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[5] A/SN. Foss Electric, Hillerød, Denmark.

[6] Thermovac Industries Corp., Copiague, Long Island, New York, U.S.A.

Chemical analysis

Dry matter and nitrogen were determined on feed, rejected feed and fecal samples by AOAC (1960) methods. Gross energy in feed, rejected feed and fecal samples was measured by combustion in a Parr oxygen bomb calorimeter. Determination of Cr_2O_3 in the Cr_2O_3 paper and fecal samples was according to the method of Christian and Coup (1954).

Volatile fatty acids in rumen samples were determined by gas-liquid chromatography (GLC) using a model 600-D Aerograph GLC with a flame ionization detector. The details are given in Appendix p. 1.

Milk fat percentage was obtained with a Milko-tester[7], milk protein by the dye-binding procedure of Udy (1956) and SNF by the plastic bead method of Golding (1964).

In preparation for fatty acid determinations the freeze-dried milk was extracted on a Goldfisch-extraction apparatus using a 1/3 (v/v) mixture of methanol and hexane. The extracted fat was transferred to a 5 ml freeze drying bulb and esterified according to the method of DeMan (1964). Concentrations of fatty acids in milk samples were determined by GLC using a model A90-P3 Aerograph GLC with a thermal conductivity type of detector. The details are given in Appendix p. 1-2.

Statistical analysis

An IBM 360/67 computer in the Department of Computing Science was used to statistically analyze all data. Program CS017 was used to calculate multiple regressions and program CS007 was used for the analysis of variance.

Duncan's new multiple range test (Steel and Torrie, 1960) was used to test differences between means. Student's t test (Steel and Torrie, 1960) was used to test differences between regressions.

[7]A/SN. Foss Electric, Hillerød, Denmark.

Results and Discussion

a. Ration effects on dietary intake

The experimental values for average daily dry matter, crude protein and gross energy consumed by cows during the digestibility studies at maximum milk production (period 1) and just before lactation termination (period 2) are summarized for each treatment in Table 5. The effects of levels of energy and roughage on the above variables for periods 1 and 2, and averages of the two periods are presented in Table 6.

It was expected that increasing allowances of energy, or of roughage within any one level of energy, would cause increased intake of dry matter or gross energy. Such results were obtained in period 2 (Table 5), but in period 1 differences were small. The apparent low intake by cows fed the high level of energy and low level of roughage was caused by one animal which went off feed during the digestion trial. If data for that animal at maximum milk production and feed intake had been omitted, no consistent differences would have existed in daily intake of dry matter or gross energy between the nine treatments.

On the average, the level of energy or of roughage had only minor and inconsistent effects on daily consumption of dry matter and gross energy in period 1 (Table 6). The apparent low intake by cows fed the low level of roughage was caused by the animal noted above which went off feed during the digestion trial.

The inconsistency and absence of major differences in dry matter and gross energy intake between treatments at the period of maximum feed intake appeared to be caused by the fact that most of the cows were consuming feed to maximum appetite and were unable to consume all of their daily rations. Results obtained in this study showed that during period 1

Table 5. Daily intake of dry matter, crude protein and gross energy, and roughage:concentrate ratios of the rations consumed for all treatments

		Treatments								
Level of energy, % of NRC requirements		90			100			120		
Level of roughage, kg/100 kg body wt		0.75	1.50	2.50	0.75	1.50	2.50	0.75	1.50	2.50
Dry matter intake, kg	Period 1	17.0	17.4	14.9	15.1	15.6	17.0	12.5	18.2	19.2
	" 2	9.8	12.0	12.9	11.9	12.9	14.9	12.8	14.6	16.0
Crude protein intake, kg	Period 1	2.6	2.6	2.5	2.5	2.4	2.8	2.2	3.1	3.3
	" 2	1.4	1.7	1.8	1.6	1.8	1.7	1.8	1.9	2.1
Gross energy intake, mcal	Period 1	73.6	75.2	65.1	64.8	66.6	75.0	55.6	81.7	84.4
	" 2	42.3	51.1	55.6	50.0	54.5	63.9	54.0	61.3	67.4
Roughage:concentrate ratio, w/w	Period 1	0.3	0.8	2.2	0.4	0.9	1.2	0.5	0.7	1.3
	" 2	0.7	2.6	23.1	0.5	2.0	7.1	0.5	1.7	3.4

Table 6. Daily intake of dry matter, crude protein and gross energy, and roughage:concentrate ratios of the rations consumed according to level of energy and roughage fed

		Level of energy, % of NRC requirements			Level of roughage, kg/100 kg body wt			Average of all cows
		90	100	120	0.75	1.50	2.50	
Dry matter intake, kg	Period 1	16.4	15.9	16.6	14.9	17.1	17.0	16.3
	" 2	11.6	13.2	14.5	11.5	13.2	14.6	13.1
	Av	14.0	14.5	15.6	13.2	15.1	15.8	
Crude protein intake, kg	Period 1	2.6	2.6	2.9	2.4	2.7	2.9	2.7
	" 2	1.6	1.7	1.9	1.6	1.8	1.8	1.8
	Av	2.1	2.1	2.4	2.0	2.3	2.4	
Gross energy intake, mcal	Period 1	71.3	68.8	73.9	64.7	74.5	74.8	71.3
	" 2	49.7	56.1	60.9	48.7	55.7	62.3	55.6
	Av	60.5	62.5	67.4	56.7	65.1	68.5	
Roughage:concentrate ratio, w/w	Period 1	1.1	0.8	0.8	0.4	0.8	1.6	0.9^{g}
	" 2	8.8	3.2	1.9	0.6	2.1	11.2	4.6^{h}
	Av	4.9	2.0	2.5	0.5^{v}	1.4^{v}	6.4^{x}	

^{vx}Values in the same row with superscripts ^{v} and ^{x} are significantly different ($P < .01$).

^{gh}Averages of all cows for the same variable with superscripts ^{g} and ^{h} are significantly different ($P < .05$).

only two cows consumed their daily allotments of feed; both of these cows were in the low energy group, one being fed the low level of roughage and the other the high level of roughage. Thus it would appear that lactating cows during the peak of milk production were unable to consume feed above the level of 90% of calculated digestible energy requirements. Presumably, as is reported by many workers (Campling et al., 1962; Conrad et al., 1964; Crampton et al., 1960; Elliott and Fokkema, 1961; Montgomery and Baumgardt, 1965), individual feed intake at the peak of production is regulated, depending on the ration dry matter digestibility, by either physical limitations of the digestive system or by physiological regulatory mechanisms.

Throughout the experimental period the cows fed high energy rations never consumed their daily allotment of feed. Animals fed the medium and low energy rations consumed their daily allotments of feed after approximately 17 and 14 weeks, respectively.

In period 2, daily intake of dry matter was affected by levels of energy and roughage in the ration. Daily intake of dry matter during the period was 11.6, 13.2 and 14.5 kg (Table 6) for the groups fed 90, 100 and 120% of NRC requirements, respectively. These results would be expected since more feed was provided for maintenance and milk production with each increase in the level of energy.

Intake of dry matter and gross energy increased with the proportion of roughage in the ration. The theoretical digestible energy values used to calculate energy allotments were 2.2 mcal per kg of roughage and 3.1 mcal per kg of concentrate. Hence, if the digestible energy supplied by the ration remained constant, a greater quantity of ration was fed when the roughage:concentrate ratio increased in this experiment.

The daily crude protein consumption varied to some extent in all treatments during both periods (Table 5). This was brought about by the different proportions of roughage and concentrate that were fed, and by the moderate changes in the crude protein percentage of the concentrates and roughages fed during the various digestibility trials.

Roughage:concentrate ratios in the rations consumed during the first period varied to some extent between treatments (Table 5). At each level of energy, the ratio increased with increasing level of roughage fed. The roughage:concentrate ratios were relatively narrow in this period, ranging from 0.3 to 2.2. Similar results were obtained in period 2, but the ratios were much wider, ranging from 0.5 to 23.1.

b. Apparent digestibility of dry matter, crude protein and gross energy

The mean values for the apparent digestion coefficients of dry matter, crude protein and gross energy in the two digestion periods are summarized for each treatment in Table 7. Mean values for each variable are shown in Table 8 for the three levels of energy and the three levels of roughage fed in both periods and for the averages of the two periods.

There were no significant differences between treatments in either period for apparent digestibility of dry matter, crude protein or gross energy (Table 7). Furthermore, level of energy or of roughage in the ration did not significantly change the apparent digestibilities of dry matter, crude protein or gross energy in the two periods (Table 8).

The non-significant differences for apparent dry matter, crude protein and gross energy digestibilities for the first period were as expected, since no major differences were noted during this period for dry matter and gross energy intakes (Tables 5 and 6). It was evident also that at maximum feed intake there was a narrow roughage:concentrate ratio

Table 7. Apparent digestion coefficients for all treatments

		Treatments								
Level of energy, % of NRC requirements		90			100			120		
Level of roughage, kg/100 kg body wt		0.75	1.50	2.50	0.75	1.50	2.50	0.75	1.50	2.50
Apparent coefficients of digestion, %										
Dry matter	Period 1	73.9	66.2	68.9	71.1	71.2	70.9	70.6	68.5	71.2
	" 2	65.1	66.1	62.1	61.2	64.0	60.6	68.2	66.5	66.9
Crude protein	Period 1	75.8	68.6	74.1	74.3	74.1	73.0	74.6	74.1	77.0
	" 2	70.6	72.0	69.8	65.8	70.6	64.6	71.8	71.8	71.0
Gross energy	Period 1	73.0	64.7	68.2	69.3	71.5	70.0	69.7	67.9	71.0
	" 2	65.5	67,0	62.2	61.4	64.5	60.5	67.8	67.7	66.1

Table 8. Apparent digestion coefficients according to level of energy and roughage fed

		Level of energy, % of NRC requirements			Level of roughage, kg/ 100 kg body wt			Average of all cows
		90	100	120	0.75	1.50	2.50	
Apparent coefficients of digestion, %								
Dry matter	Period 1	69.7	71.1	70.1	71.9	68.6	70.3	70.3[e]
	" 2	64.4	61.9	67.2	64.8	65.6	63.2	64.5[f]
	Av	67.0	66.5	68.7	68.4	67.1	66.8	
Crude protein	Period 1	72.8	73.8	75.2	74.9	72.3	74.7	74.0[g]
	" 2	70.8	67.0	71.5	69.4	71.5	68.5	70.0[h]
	Av	71.8	70.4	73.4	72.2	71.9	71.6	
Gross energy	Period 1	68.6	70.2	69.5	70.6	68.0	69.7	69.5[e]
	" 2	64.9	62.2	67.2	64.9	66.4	63.0	64.8[f]
	Av	66.8	66.2	68.4	67.8	67.2	66.4	

[efgh] Averages of all cows for the same variable with superscripts [e] and [f] are significantly different ($P < .01$); and [g] and [h] ($P < .05$).

ranging from 0.3 to 2.2 in the experimental rations consumed (Table 5). Since all cows were eating to maximum appetite and there was a narrow range in roughage:concentrate ratios, no appreciable differences would be anticipated in the apparent dry matter, crude protein and gross energy digestion coefficients.

On the other hand, the apparent dry matter, crude protein and gross energy digestibilities in period 2 were expected to decrease as the level of energy in the daily ration increased. It was calculated that dry matter intake increased from 1.6 to 2.0 times maintenance between the groups fed 90 and 120% of NRC requirements, but there were no appreciable differences between coefficients of digestibility (Table 8). It was also expected that increasing the level of roughage in the ration, in this period, would cause a decrease in digestion coefficients. Although the level of roughage fed increased from 0.75 to 2.50 kg/100 kg body weight, resulting in an increase in the roughage:concentrate ratios of the ration consumed from 0.6 to 11.2 (Table 6), there were no appreciable effects on coefficients of digestibility (Table 8). The effects of level of energy (dry matter intake) and roughage fed on digestibility are contrary to concepts proposed by Flatt et al. (1966) and Moe et al. (1965).

Digestion coefficients for dry matter, crude protein and gross energy were lower in period 2 than in period 1 for most of the treatments (Table 7) and for all levels of energy and roughage (Table 8). As a result, significant differences were obtained between the average values of all animals in each period (Table 8). There is no apparent explanation for the decrease in digestibility between periods 1 and 2. Feed intake was lower and the proportion of roughage in the ration was higher in period 2 than in period 1; however, neither feed intake nor level of roughage appeared to

affect digestion coefficients within period 2. Therefore, it seems evident from these experimental results that in addition to the influence of level of intake and ration composition on digestibility (Flatt et al., 1966; Moe et al., 1965; Putnam and Loosli, 1959), other physiological factors may be involved.

c. Rumen VFA and rumen pH

The average values for rumen VFA and rumen pH at maximum milk production (period 1) and just before lactation termination (period 2) are summarized for each treatment in Table 9. The effects of levels of energy and levels of roughage in the ration on these variables in both periods and the average of the two periods are shown in Table 10.

Differences between treatments in total VFA, pH or proportions of VFA in rumen liquor (Table 9) were often marked but no consistent trends were evident.

Level of energy or roughage in the ration did not have appreciable effects on total VFA in rumen liquor in either period (Table 10). However, total VFA were slightly lower in period 2 than in period 1, and significantly lower ($P < .01$) when the averages for all cows in each period were compared. On the average, samples taken in period 1 contained 13.8 mmoles/100 ml as compared to 12.2 mmoles/100 ml in samples obtained during period 2. This might be expected since average daily gross energetic intake was 71 mcal daily in period 1 and 56 mcal daily in period 2.

Significant changes ($P < .01$) in the average pH of rumen contents of all animals occurred between samples at the peak of milk production and those prior to the termination of lactation (Table 10). Rumen pH increased from a low of 6.52 at the peak of milk production to 6.83 before cessation of the experimental period. In general, the rumen pH obtained in this study

Table 9. Rumen VFA and rumen pH for all treatments

		Treatments								
Level of energy, % of NRC requirements		90			100			120		
Level of roughage, kg/100 kg body wt		0.75	1.50	2.50	0.75	1.50	2.50	0.75	1.50	2.50
Total rumen VFA,	Period 1	13.7	12.0	15.6	13.4	12.7	15.0	13.4	12.3	16.6
mmoles/100 ml	" 2	13.2	9.9	14.6	12.9	11.2	10.5	10.7	13.0	13.8
Rumen pH	Period 1	6.3	6.8	6.7	6.4	6.6	6.5	6.5	6.7	6.3
	" 2	6.5	7.1	7.0	6.4	7.1	8.9	6.9	6.6	6.9
Molar proportions, %										
Acetate	Period 1	54.0	61.7	64.3	55.5	58.7	61.1	61.4	57.0	61.1
	" 2	65.2	66.8	68.3	66.4	66.8	69.9	62.9	62.3	67.0
Propionate	Period 1	31.7	23.8	20.5	34.9	28.1	24.6	20.3	28.7	22.5
	" 2	15.2	18.4	18.5	18.1	18.0	16.6	22.4	24.9	19.4
iso-Butyrate	Period 1	0.6	0.6	0.7	0.5	0.6	0.5	0.9	0.6	0.6
	" 2	1.0	1.0	0.9	0.7	0.6	0.5	0.6	0.6	0.6
n-Butyrate	Period 1	11.2	12.1	12.2	7.0	10.2	11.6	13.7	10.7	13.5
	" 2	13.8	11.3	10.2	12.6	12.4	11.5	11.7	10.5	11.3
iso-Valerate	Period 1	0.4	0.5	0.7	0.6	0.7	0.5	1.4	1.0	0.7
	" 2	1.1	1.0	0.8	0.7	0.9	0.5	1.0	0.6	0.6
n-Valerate	Period 1	2.2	1.3	1.6	1.6	1.8	1.8	2.0	2.0	1.6
	" 2	1.5	1.5	1.4	1.5	1.4	1.1	1.4	1.2	1.1
Acetate:Propionate ratio	Period 1	1.7	2.6	3.1	1.6	2.1	2.5	3.1	2.0	2.8
	" 2	3.7	3.6	3.7	3.7	3.7	4.2	2.8	2.5	3.5

Table 10. Rumen VFA and rumen pH according to level of energy and roughage fed.

		Level of energy, % of NRC requirements			Level of roughage, kg/100 kg body wt			Average of all cows
		90	100	120	0.75	1.50	2.50	
Total rumen VFA,	Period 1	13.7	13.7	14.1	13.5	12.3	15.7	13.9[e]
mmoles/100 ml	" 2	12.6	11.5	12.5	12.2	11.4	13.0	12.2[f]
	Av	13.2	12.6	13.3	12.9	11.9	14.4	
Rumen pH	Period 1	6.6	6.5	6.5	6.4	6.7	6.5	6.5[e]
	" 2	6.9	6.8	6.8	6.6	7.0	6.9	6.8[f]
	Av	6.7	6.7	6.6	6.5	6.8	6.7	
Molar proportions, %								
Acetate	Period 1	60.0	58.4	59.9	57.1	59.1	62.2	59.4[g]
	" 2	66.8	67.7	64.1	64.9	65.4	68.4	66.2[h]
	Av	63.4	63.1	62.0	61.0[v]	62.2[y]	65.3[xz]	
Propionate	Period 1	25.3	29.2	23.9	29.0	26.9	22.5	26.1[e]
	" 2	18.1	17.5	22.2	19.3	20.4	18.1	19.3[f]
	Av	21.7	23.4	23.0	24.2	23.6	20.3	
iso-Butyrate	Period 1	0.6	0.5	0.7	0.7	0.6	0.6	0.6
	" 2	1.0	0.6	0.6	0.8	0.7	0.7	0.7
	Av	0.8[c]	0.6[d]	0.7	0.7	0.7	0.6	
n-Butyrate	Period 1	11.8	9.6	12.6	10.6	11.0	12.4	11.4
	" 2	11.8	12.2	11.1	12.7	11.4	11.0	11.7
	Av	11.8	10.9	11.9	11.6	11.2	11.7	
iso-Valerate	Period 1	0.5	0.6	1.0	0.8	0.7	0.6	0.7
	" 2	1.0	0.7	0.7	0.9	0.8	0.6	0.8
	Av	0.8	0.6[c]	0.9[d]	0.9	0.8	0.6	
n-Valerate	Period 1	1.7	1.7	1.9	1.9	1.7	1.7	1.8[g]
	" 2	1.5	1.3	1.3	1.5	1.3	1.2	1.3[h]
	Av	1.6	1.5	1.6	1.7	1.5	1.4	
Acetate:Propionate	Period 1	2.5	2.1	2.6	2.1	2.2	2.8	2.4[e]
ratio	" 2	3.7	3.9	2.9	3.4	3.2	3.8	3.5[f]
	Av	3.1	3.0	2.8	2.8	2.8	3.3	

[cd] Values in the same row with superscripts [c] and [d] are significantly different ($P < .05$).

[vxyz] Values in the same row, under roughage heading, with superscripts [v] and [x] are significantly different ($P < .01$); [y] and [z] ($P < .05$).

[efgh] Averages of all cows for the same variable with superscipts [e] and [f] are significantly different ($P < .01$); [g] and [h] ($P < .05$).

was lower as the total amount of VFA in the rumen liquor increased. Hibbs et al. (1956) also demonstrated that pH was inversely proportional to VFA concentrations in the rumen.

The average molar proportions of acetate, propionate, n-butyrate and n-valerate in rumen fluid were not significantly affected by the level of energy in the ration. Significant differences ($P < .05$) did occur between the two-period average for iso-butyrate and iso-valerate molar proportions for the low and medium, and medium and high levels of energy nutrition, respectively. However, these acids contributed only a very small percentage to the total VFA in the rumen fluid and their significance in intermediary metabolism is largely unknown.

The level of roughage in the ration had significant effects on the proportions of some of the VFA in rumen fluid. When data for the two periods were averaged, the proportion of acetate increased significantly when the level of roughage increased from low to high ($P < .01$) and from medium to high ($P < .05$). There was also a significant increase ($P < .05$) in acetate between periods 1 and 2 when data for all the cows were averaged. Propionate decreased when acetate increased but significant differences ($P < .01$) were detected only between averages for all the cows in periods 1 and 2. Similar results for acetate and propionate have been reported by Balch et al. (1955), Brown et al. (1962b), Ensor et al. (1959), Shaw and Ensor (1959), Shaw et al. (1959) and Stanley et al. (1964), who found that an increase in the proportion of roughage in the ration elevated the molar proportion of rumen acetate and decreased the production of propionate. In general, it was also found in this study that an increase in the proportion of roughage in the ration resulted in a decreasing trend in the average molar proportions of iso-butyrate, iso-valerate and n-valerate, and no change in n-butyrate.

The daily rations fed in the experiment contained from 4.1 to 12.2 kg of roughage plus sufficient concentrates to provide the calculated energy requirements. Although, the proportion of roughage did affect VFA concentrations in the rumen contents to some extent, the level of roughage in the rations may not have been low enough to produce major effects in VFA proportions in the rumen fluid.

d. Milk production and composition of the milk

Comparisons of milk production are given only for the first 28 weeks of the lactation. Three cows were removed from the experiment by that time so that statistical analysis beyond that period was not possible. Two cows were dried off prior to 28 weeks on the experiment, one at 20 weeks and one at 25 weeks, and their production during the last week was used to complete analysis to 28 weeks.

The experimental values for average daily milk, FCM and SCM produced, and percentages of fat, protein and SNF in the milk are summarized for each treatment in Table 11. The specific effects of levels of energy and levels of roughage in the ration on daily milk, FCM and SCM produced, and percentages of fat, protein and SNF are presented in Table 12.

Milk production

There was some variation in milk production between treatments (Table 11), but no consistent trends to increasing or decreasing production were noted with increasing level of energy or increasing level of roughage within different levels of energy. Lowest daily production was obtained with cows fed the low level of energy with the high level of roughage; highest daily production was obtained with cows fed the medium level of energy and the high level of roughage. However, no significant differences were detected.

Table 11. Average daily milk production and milk composition during the first 28 weeks of lactation for all treatments

	Treatments								
Level of energy, % of NRC requirements	90			100			120		
Level of roughage, kg/100 kg body wt	0.75	1.50	2.50	0.75	1.50	2.50	0.75	1.50	2.50
Milk, kg	20.5	23.1	16.2	21.9	19.3	24.7	20.2	21.7	19.2
FCM, kg	18.2	20.9	14.6	19.2	16.4	23.6	18.0	19.2	18.1
SCM, kg	18.4	21.2	14.8	20.0	16.8	24.2	18.3	19.9	18.6
Fat, %	3.18	3.39	3.31	3.25	3.09	3.69	3.24	3.26	3.63
Protein, %	3.41	3.10	3.22	3.44	3.30	3.44	3.66	3.60	3.74
SNF, %	8.88	8.83	8.79	9.16	8.85	9.10	8.93	9.11	9.14

Table 12. Average daily milk production and milk composition during the first 28 weeks of lactation according to level of energy and roughage fed

	Level of energy, % of NRC requirements			Level of roughage, kg/100 kg body wt			Average of all cows
	90	100	120	0.75	1.50	2.50	
Milk, kg	20.0	22.0	20.4	20.9	21.4	20.0	20.8
FCM, kg	17.9	19.7	18.4	18.5	18.8	18.8	18.7
SCM, kg	18.1	20.3	18.9	18.9	19.3	19.2	19.1
Fat, %	3.29	3.34	3.38	3.22^{v}	3.25^{v}	3.54^{x}	3.34
Protein, %	3.24^{a}	3.39^{ab}	3.67^{b}	3.50^{v}	3.33^{x}	3.47^{v}	3.43
SNF, %	8.83^{a}	9.04^{b}	9.06^{b}	8.99	8.93	9.01	8.98

^{ab}Values in the same row, under energy heading, with superscripts ^{a} and ^{b} are significantly different ($P < .01$).

^{vx}Values in the same row, under roughage heading, with superscripts ^{v} and ^{x} are significantly different ($P < .01$).

In general, there was little difference between FCM and SCM, although SCM values were slightly higher. Correction of milk production for content of SNF did not appear to cause any appreciable change in values already corrected for the fat content of the milk.

No significant differences were noted in daily milk, FCM or SCM production between cows fed different levels of energy or different levels of roughage (Table 12). However, highest average production was obtained when cows were fed energy at 100% of NRC requirements.

There was a significant interaction between level of energy in the ration and stage of lactation for milk ($P < .01$), FCM ($P < .05$) and SCM ($P < .05$) production. The interaction was the result of the decline in FCM produced by cows fed 90% of their calculated energy requirements (Fig. 8). Up to the 12th week of lactation these cows had an average production of FCM comparable to that of cows fed the two higher levels of energy; after the 12th week of lactation, FCM production by cows fed the low level of energy declined faster than by cows fed the higher levels of energy and significantly faster after the 16th week.

On the average, cows milked 41, 41 and 34 weeks when fed high, medium and low levels of energy. Lowest average production was obtained in cows fed the high level of roughage and the low level of energy; these cows milked only 27 weeks on the average, as compared to 37 weeks by cows fed this level of energy with the medium and low levels of roughage.

It would appear that cows fed the low level of energy were able to draw on reserves of body fat early in lactation to achieve high levels of production. However, body reserves were rapidly depleted and the intake of dietary nutrients was insufficient to maintain milk production, resulting in the rapid decline in the average daily milk produced after 12 weeks of

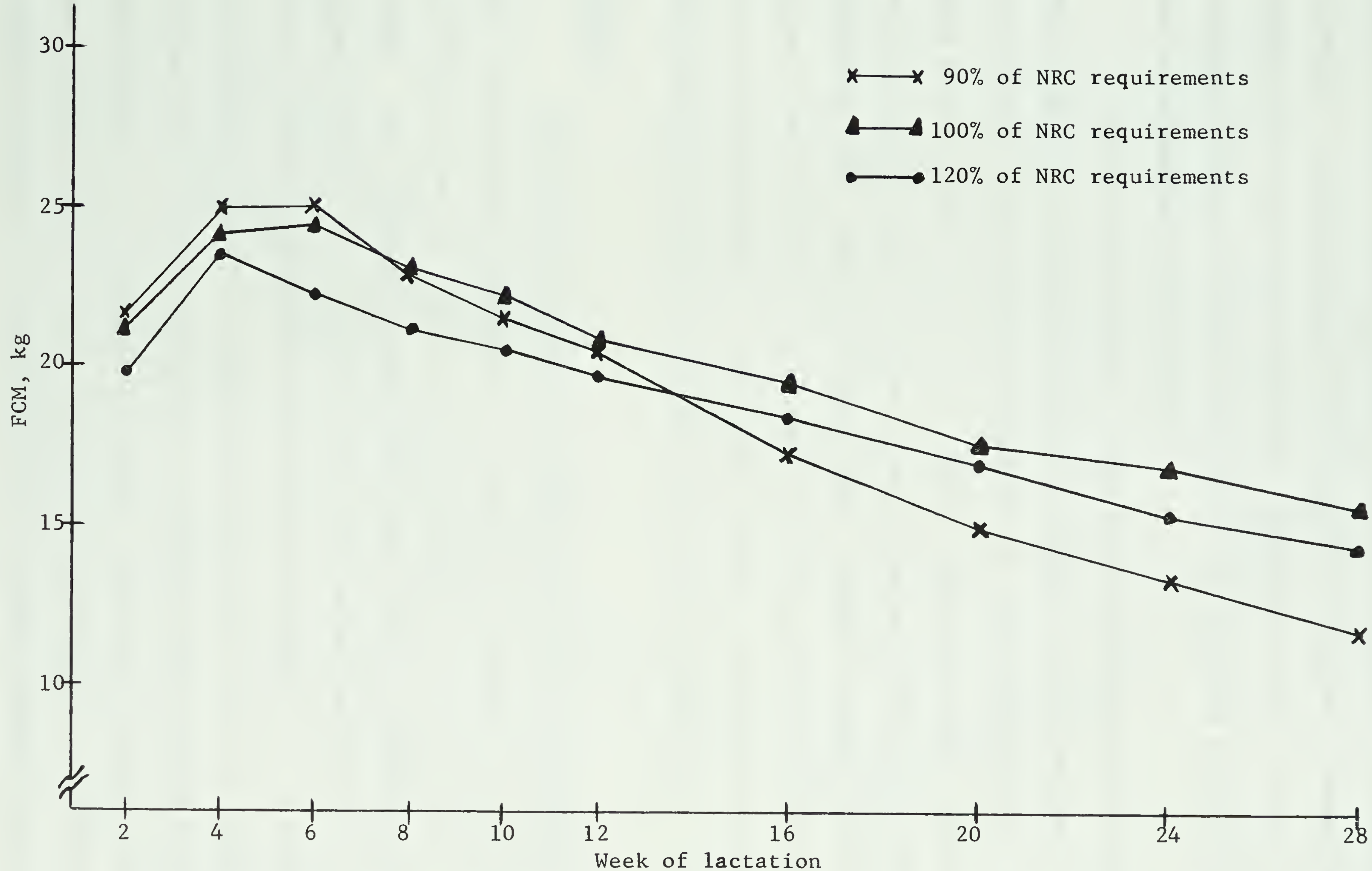

Fig. 8. The effect of level of energy in the ration on variation in FCM production during the lactation.

lactation. Cows fed the medium and high levels of energy also used body fat reserves in early lactation when they could not consume all of their ration allotments. As milk production and the daily feed given to these cows declined to the level where they could consume all or most of their rations, the intake of dietary nutrients was equal to or in excess of their requirements for maintenance and production.

While in some reports, it has been noted that elevated levels of energy nutrition or increased density of energy in the ration gave a significant increase in milk production (Balch et al., 1961; Bishop et al., 1963; Castle et al., 1959 ; Morris et al., 1958), other reports have indicated no beneficial effects from increases in energy levels and/or concentration (Boyd and Mathew, 1962; Rumery and Plum, 1963). Elliot and Loosli (1959) fed rations in which the level of estimated net energy (ENE) intake above maintenance was held constant. They noted no change in FCM production with rations containing 40, 60 or 80% of the ENE in the form of concentrates.

Milk fat

When 0.75 or 1.50 kg of roughage per 100 kg of body weight was fed daily in this study, irrespective of the level of energy, the percentage milk fat (Table 12) was 0.3 lower ($P < .01$) than when the 2.50 kg level of roughage was fed. Fig. 9 illustrates the fitted lines from the regression equations (Appendix Table 2) for variation in the daily percentage milk fat during lactation between cows fed the three levels of roughage. It is evident from this illustration that the significant differences in milk fat between the two lower levels and the high level of roughage intake occurred from the beginning until the 24th week of lactation. After the 24th week, all rations showed somewhat similar effects on milk fat percentages. As milk production declined the amount of concentrates fed to the cows decreased, resulting in

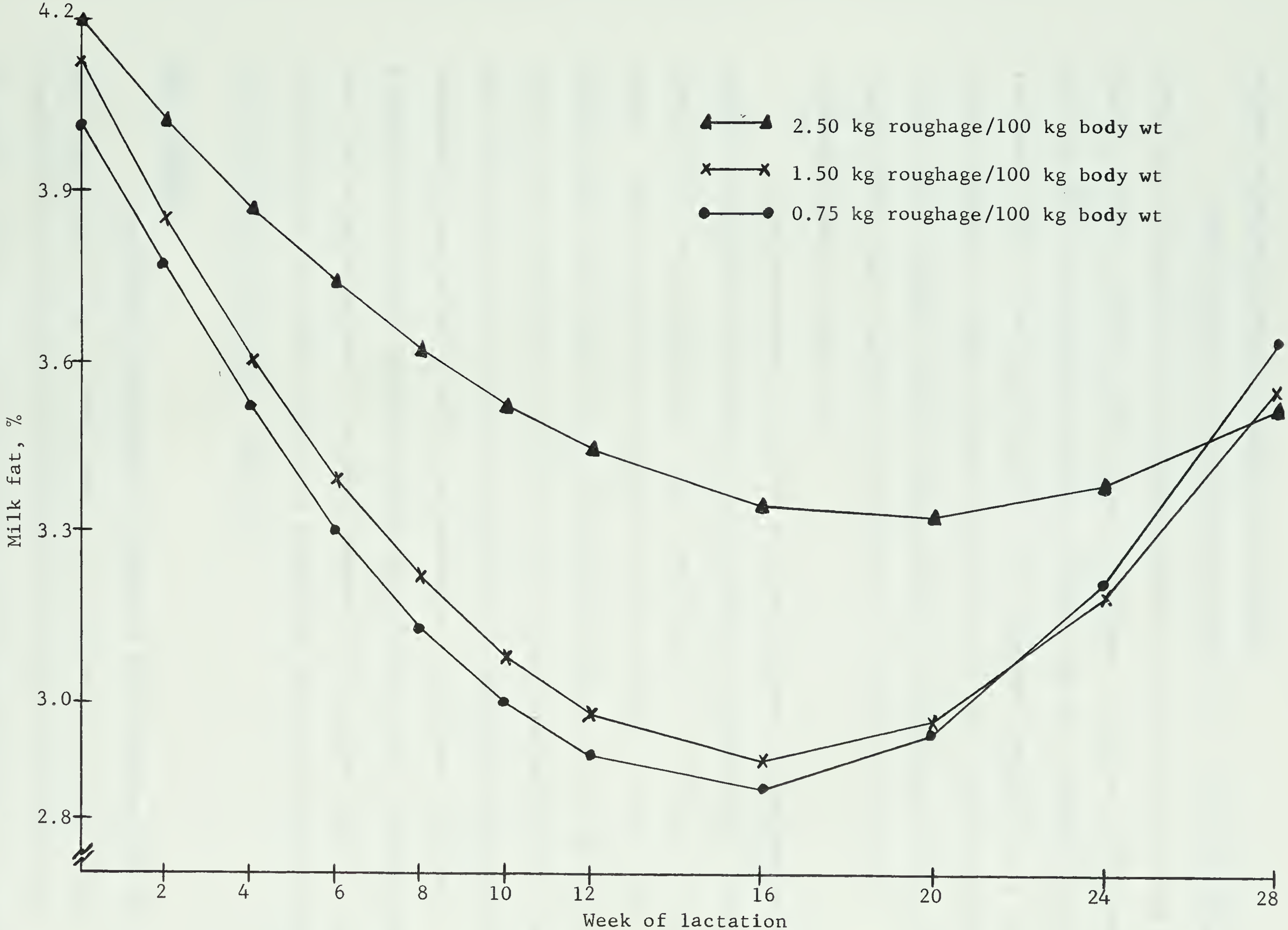

Fig. 9. The effect of level of roughage in the ration on variation in percentage milk fat during the lactation.

higher roughage:concentrate ratios in all rations.

Recent reports in the literature (Emery et al., 1964; Leighton, 1965) have indicated similar decreasing effects on the fat test of milk by feeding rations containing 20% or less of roughage. Leighton (1965) observed that the milk fat percentage returned to normal when the roughage level was increased to 30%. The data obtained in this experiment indicate there was little difference in milk fat when cows were fed rations containing between 32 and 59% of roughage (low and medium levels), but percentage milk fat was significantly lower than when the proportion of roughage in the ration increased markedly (high level).

The phenomenon of milk fat depression appears to be complex and cannot be explained on the basis of low intake of roughage alone. Kesler and Spahr (1964) have suggested that fiber levels below 13 to 14% may be detrimental. Their results are in agreement with the findings of Balch and Rowland (1957) that when rations containing low amounts of hay are fed, the nature of the concentrates and starch content determines whether that diet will affect milk fat per cent. Many explanations involving changes in rumen fermentation and volatile fatty acid production in the rumen have been suggested as the cause for low-fat milk, when rations high in concentrates were fed to lactating cows. The data obtained in this study showed a decrease in the milk fat test when there was a significant decrease in rumen acetate and an increase in propionate (Table 10).

<u>Milk protein</u>

The average percentages of protein in milk from cows fed low, medium and high levels of energy were 3.24, 3.39 and 3.67, respectively, (Table 12). Each increase in percentage protein was highly significant ($P < .01$). To illustrate the variation in milk protein during the lactation

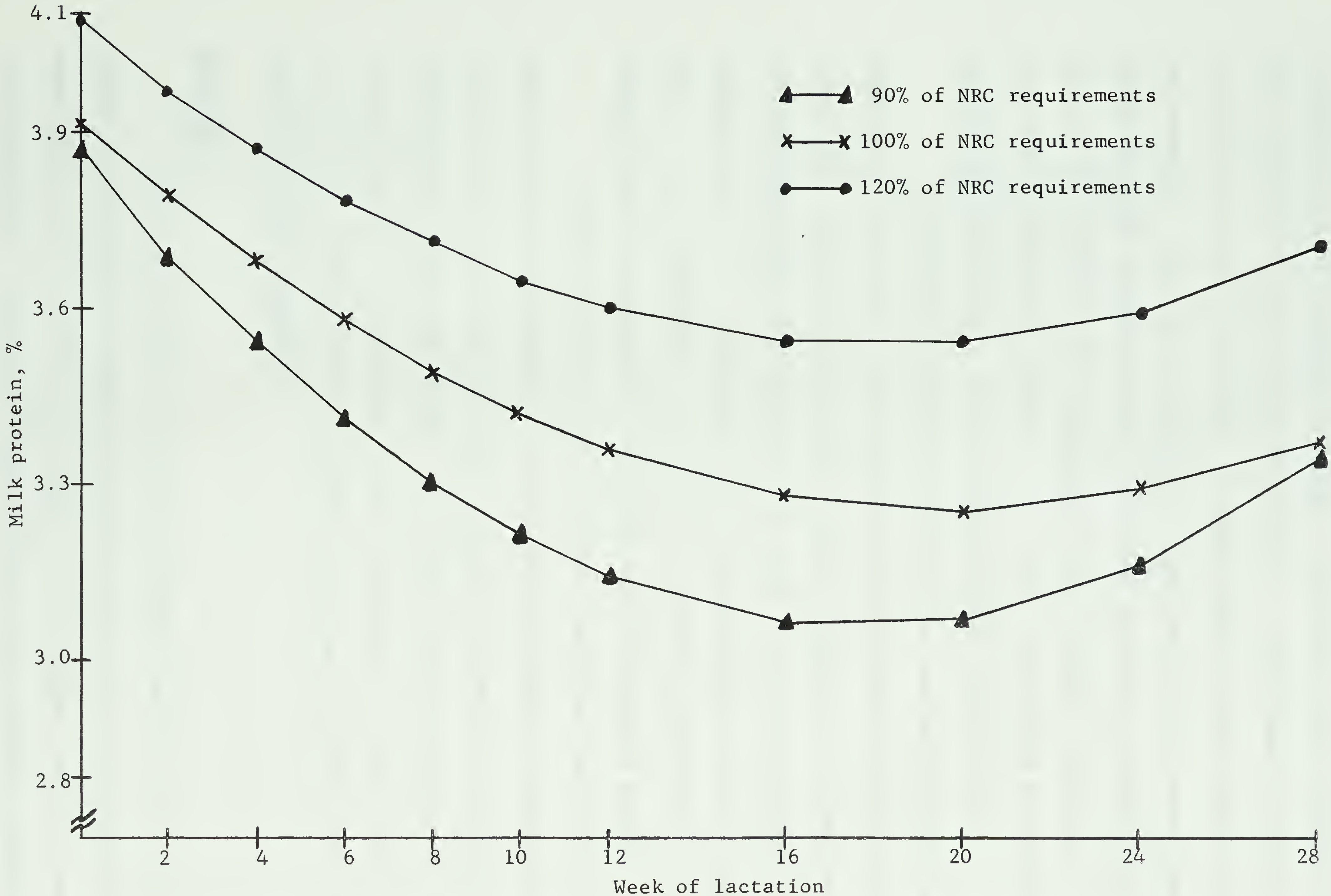

Fig. 10. The effect of level of energy in the ration on variation in percentage milk protein during the lactation.

of cows fed the three levels of energy, fitted lines were computed (Fig. 10) from regression equations (Appendix Table 2). Differences in protein content of the milk for the three levels of energy nutrition were similar throughout the lactation. The average increases in per cent of milk protein over the entire lactation associated with increasing the level of energy from 100 to 120% and 90 to 120% of NRC requrements were 8 and 13%, respectively. Similar results were reported by Becker et al. (1965), who noticed a 10% decline in the milk protein content, after reducing the TDN intake by 25%.

A significant change ($P < .01$) in per cent milk protein was also observed in the present study between cows fed different levels of roughage (Table 12). Animals fed the medium level of roughage had a lower milk protein percentage throughout the lactation than those fed the high and low levels of roughage (Fig. 11). The fitted lines (from regression equations, Appendix Table 2) vary considerably, resulting in a different pattern for the low roughage group as compared to the medium and high roughage groups.

Several reports in the literature postulate that higher levels of propionic acid are produced in the rumen by increasing the level of concentrate in the ration, and this might indirectly increase the production of milk proteins (Huber and Boman, 1966; Rook, 1959; Rook and Storry, 1964). An increased flow of propionate to the liver seems to cause a 'sparing effect' on blood amino acids, leaving a greater percentage available to the mammary gland for protein synthesis.

<u>Milk SNF</u>

Per cent of SNF in the milk increased ($P < .01$) when the level of energy in the ration increased from 90% to either 100 or 120% of NRC requirements (Table 12); the difference between the two higher levels of energy was not significant. Variations in per cent SNF during lactation between cows

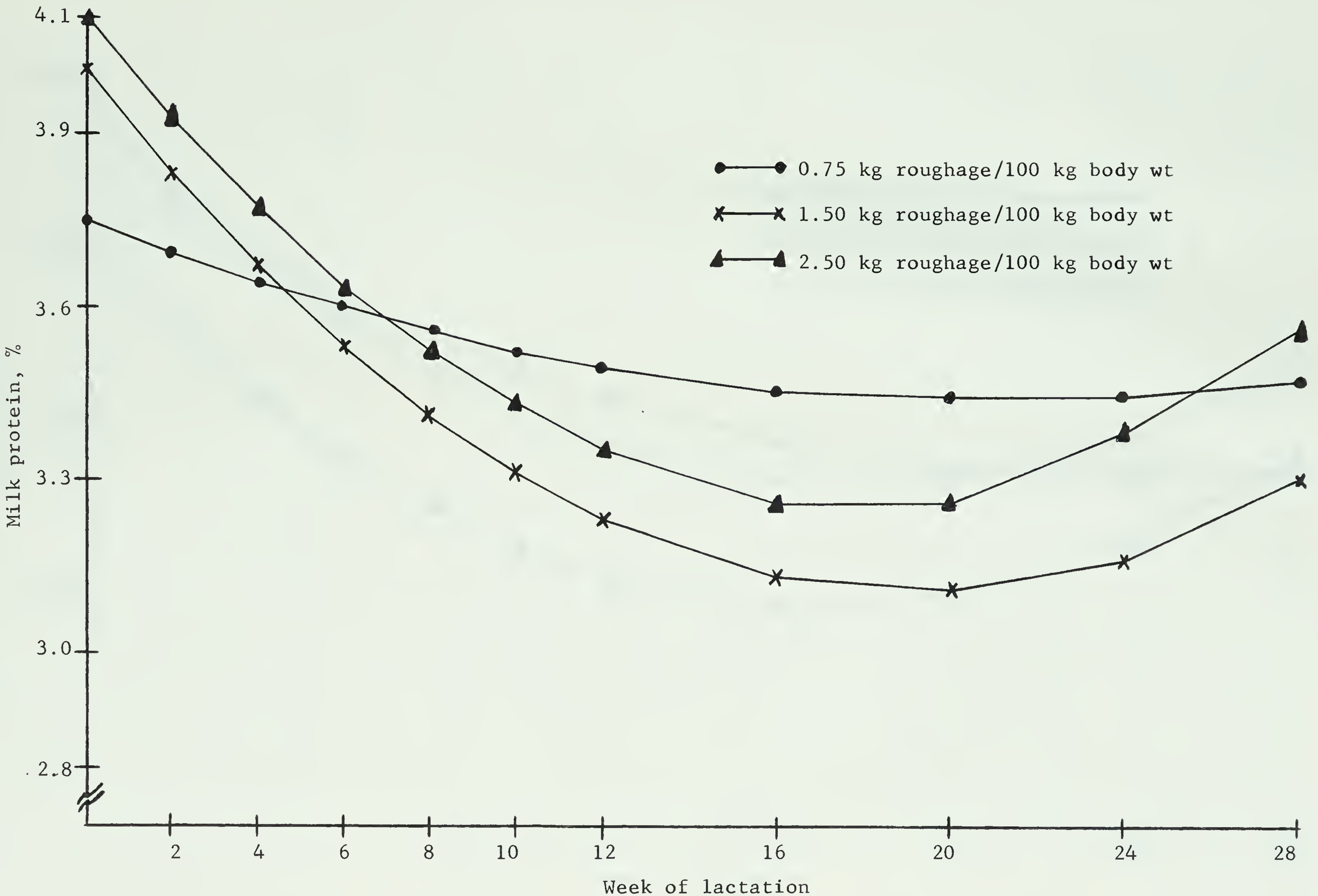

Fig. 11. The effect of level of roughage in the ration on variation in percentage milk protein during the lactation.

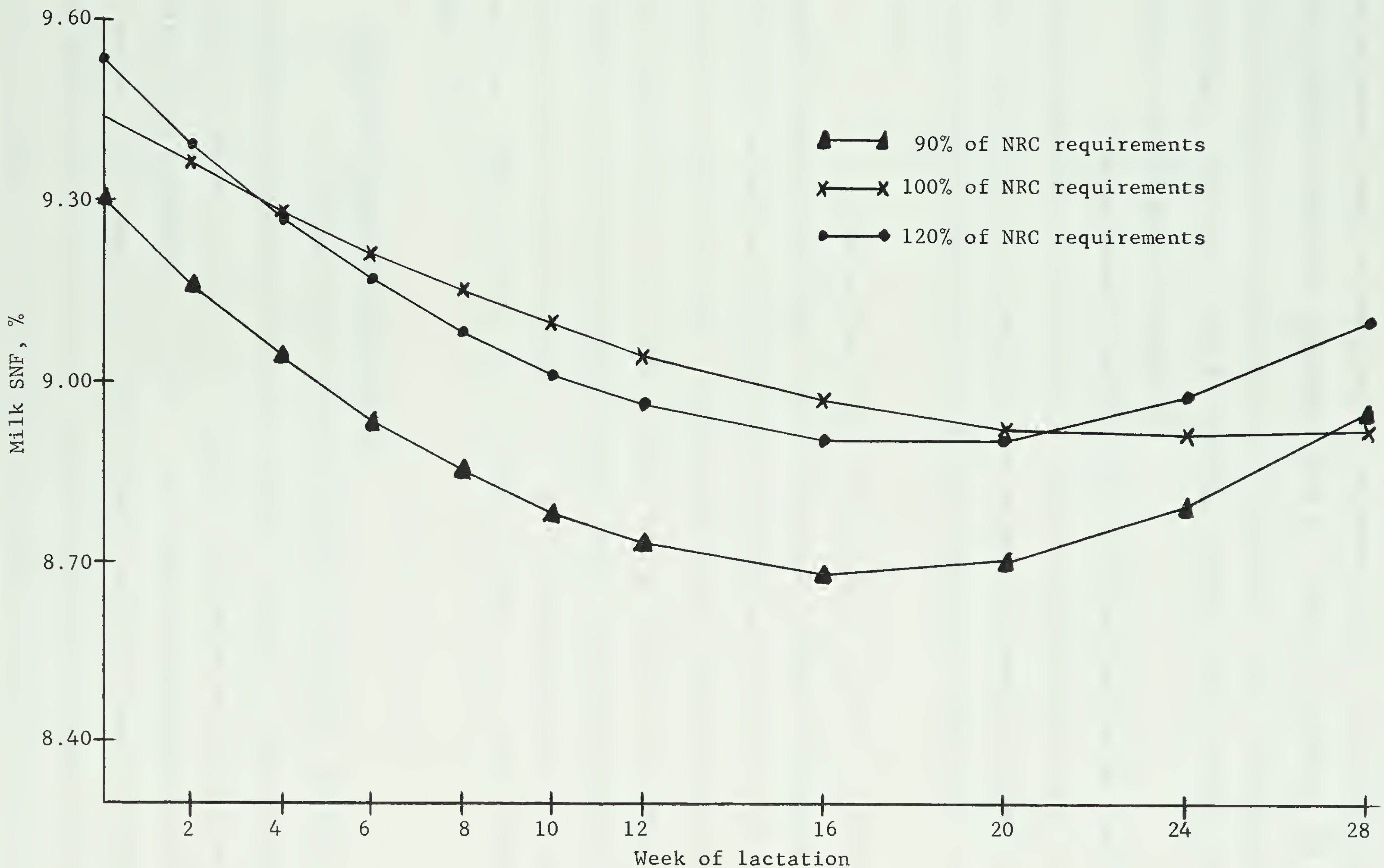

Fig. 12. The effect of level of energy in the ration on variation in percentage milk SNF during the lactation.

fed different levels of energy are illustrated in Fig. 12 by fitted lines from regression equations (Appendix Table 2). Stage of lactation did not substantially alter the variation; cows fed the low level of energy produced milk lower in SNF than cows fed the medium and high levels of energy throughout the first 28 weeks of lactation.

A large number of studies have indicated that the per cent SNF increased when the plane of energy intake was elevated (Balch et al., 1961; Burt, 1957;Holmes et al.,1960),in other reports no beneficial effects due to increased energy intake were found (Bernett and Olson, 1963; Brown et al., 1962b).

It is apparent that most of the increase in SNF is in the protein fraction (Murdock and Hodgson, 1962); in several instances (Bishop et al., 1963; Boyd and Mathew, 1962) there was a significant increase in per cent protein but not in total SNF. Similar results were obtained in the present study where per cent milk protein and SNF varied 0.4 and 0.2 , respectively, between the low and high levels of energy intake. This would suggest that per cent lactose and/or minerals in the milk declined when higher energy levels were fed, since there was a greater increase in per cent milk protein than in SNF.

e. Fatty acid composition of milk fat

The average values obtained throughout lactation for fatty acids in milk fat are presented for each treatment in Table 13. Data showing the effects of levels of energy and roughage in the ration on the fatty acids in milk fat are summarized in Table 14. All values are expressed as a per cent by weight of the total fatty acids measured. Fig. 13-15 and 17-20 illustrate the fitted lines calculated from regression equations (Appendix Tables 3-6) for all milk fatty acids which showed significant differences during the

Table 13. Average values of the fatty acids in milk fat during the first 28 weeks of lactation for all treatments

	Treatments								
Level of energy, % of NRC requirements	90			100			120		
Level of roughage, kg/100 kg body wt	0.75	1.50	2.50	0.75	1.50	2.50	0.75	1.50	2.50
Fatty acids, wt %									
4:0	4.03	4.33	4.34	4.06	3.85	4.42	3.31	4.15	4.14
6:0	2.36	2.52	2.27	2.42	2.15	2.58	2.00	2.47	2.54
8:0	1.43	1.52	1.30	1.57	1.32	1.63	1.31	1.62	1.64
10:0	3.20	3.29	2.68	3.39	2.82	3.36	3.22	3.63	3.65
12:0	3.57	3.47	2.89	3.63	3.19	3.53	3.98	4.05	3.98
14:0	11.98	12.33	11.11	11.56	11.16	11.97	12.53	12.86	12.75
16:0	29.26	29.98	29.23	26.70	28.09	27.92	31.43	28.48	30.02
18:0	11.93	11.95	10.93	12.03	11.80	11.60	9.65	11.09	11.86
18:1	27.44	25.78	29.51	28.66	29.31	27.37	27.25	26.46	24.20
18:2	3.05	3.17	3.03	3.90	3.71	3.26	3.73	3.15	3.20
18:3	1.72	1.74	2.69	2.07	2.51	2.37	1.58	2.04	2.01

Table 14. Average values of the fatty acids in milk fat during the first 28 weeks of lactation according to level of energy and roughage fed

	Level of energy, % of NRC requirements			Level of roughage, kg/100 kg body wt			Average of all cows
	90	100	120	0.75	1.50	2.50	
Fatty acids, wt %							
4:0	4.23	4.11	3.87	3.80^{vy}	4.11^{z}	4.30^{x}	4.70
6:0	2.38	2.38	2.34	2.26^{y}	2.38	2.46^{z}	2.37
8:0	1.42^{c}	1.51^{d}	1.52^{d}	1.44	1.48	1.52	1.48
10:0	3.06^{a}	3.19^{a}	3.50^{b}	3.27	3.25	3.23	3.25
12:0	3.31^{a}	3.45^{a}	4.00^{b}	3.73	3.57	3.47	3.59
14:0	11.77^{a}	11.56^{a}	12.71^{b}	12.02	12.08	11.94	12.01
16:0	29.49^{a}	27.57^{b}	29.98^{a}	29.13	28.85	29.06	29.01
18:0	11.60^{c}	11.81^{c}	10.87^{d}	11.20	11.61	11.46	11.42
18:1	27.58^{c}	28.45^{a}	25.97^{bd}	27.78	27.18	27.03	27.33
18:2	3.08	3.62	3.36	3.56^{y}	3.34	3.16^{z}	3.35
18:3	2.05^{c}	2.32^{d}	1.88^{c}	1.79^{vy}	2.10^{z}	2.36^{xy}	2.08

^{abcd}Values in the same row, under energy heading, with superscripts ^{a} and ^{b} are significantly different ($P < .01$); ^{c} and ^{d} ($P < .05$).

^{vxyz}Values in the same row, under roughage heading, with superscripts ^{v} and ^{x} are significantly different ($P < .01$); ^{y} and ^{z} ($P < .05$).

lactation due to either level of energy or level of roughage in the ration. The effects of level of energy in the ration on change in body weight and percentage fat in the milk produced during the lactation are illustrated in Fig. 16 and 18, respectively, from the fitted lines calculated from regression equations (Appendix Tables 4 and 5).

The major components of butterfat (Table 13) were 14:0, 16:0, 18:0 and 18:1 fatty acids with average percentages of 12.0, 29.0, 11.4 and 27.3, respectively, of the total fatty acids measured. The 4:0, 6:0, 8:0, 10:0, 12:0, 18:2 and 18:3 fatty acids were present in amounts varying from 1.3 to 4.4%. In general these observations are in agreement with the results of Jorgensen et al. (1965).

The level of energy in the ration in this study significantly affected the level of many of the fatty acids in the butterfat (Table 14). The 8:0, 10:0, 12:0 and 14:0 acids were significantly higher in butterfat from cows fed high energy rations, whereas the 18:0, 18:1 and 18:3 fatty acids were significantly lower. On the average, high energy rations favoured production of short chain fatty acids.

The variation in milk fatty acid fractions due to level of energy in the ration may be attributable largely to the marked differences in loss of body weight by cows fed the three levels of energy. Change in body weight indicated that weight losses were more pronounced for cows fed the low and medium levels of energy (Table 16, page 82) than for those fed the high level of energy. Loss in body weight would have been associated with mobilization of body fat. As a result the main components of tallow (body fat), 16:0, 18:0 and 18:1 acids, would occur in the blood stream in greater quantities during body fat mobilization and increase the proportion of long-chain fatty acids in milk fat.

The 8:0, 10:0, 12:0 and 14:0 milk fatty acids were found to be markedly higher (Fig. 13 and 14) during the first half of lactation for the cows fed the high level of energy. By contrast, during the same period the 18:1 fraction was substantially lower for the cows fed the high level of energy (Fig. 15). This could have been the result of lower body weight losses recorded for the cows fed the high level of energy as compared with weight losses by cows fed the low and medium levels of energy during the first half of the experimental period (Fig. 16). Brown et al. (1962a) noted that low roughage rations supplemented with beef tallow lowered the 10:0, 12:0 and 14:0 fractions and increased the 18:1 and 18:3 fractions. It seems evident that similar effects on milk fatty acids could be obtained either by adding beef tallow to the ration or by the mobilization of body fat.

In general, the 8:0 and 18:3 acids did not appear to change markedly as a percentage of the total amount of fatty acids measured during the experimental period (Fig. 13 and 15). On the average 10:0, 12:0, 14:0 and 16:0 fatty acids increased during the first 16 weeks of lactation and remained fairly stable thereafter (Fig. 13, 14 and 17); thus emphasizing the smaller contribution by body fat to the production of milk fatty acids as lactation progressed. As a result, fewer 18 carbon fatty acids would be produced, and the proportion of shorter chain acids would increase resulting in higher levels of these acids as a percentage of the total butterfat. In fact the 18:1 fraction decreased markedly during the first 16 weeks of lactation with very little change during the remainder of the period.

The 4:0 and 18:0 fractions were consistently lower throughout the lactation (Fig. 18) when cows were fed the high energy ration than when cows consumed the rations containing the lower levels of energy. These acids

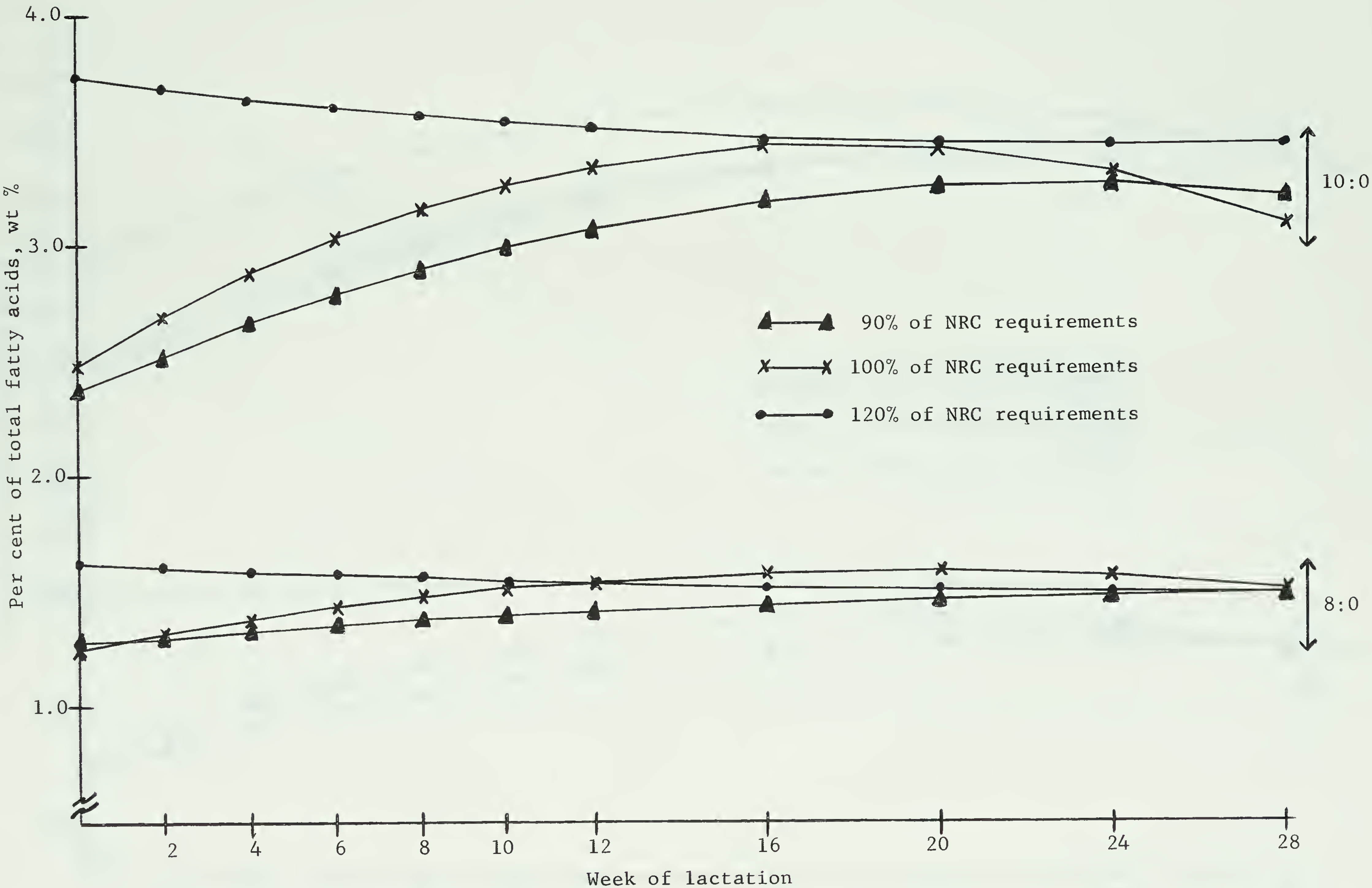

Fig. 13. The effect of level of energy in the ration on variation in percentage 8:0 and 10:0 fatty acids during the lactation.

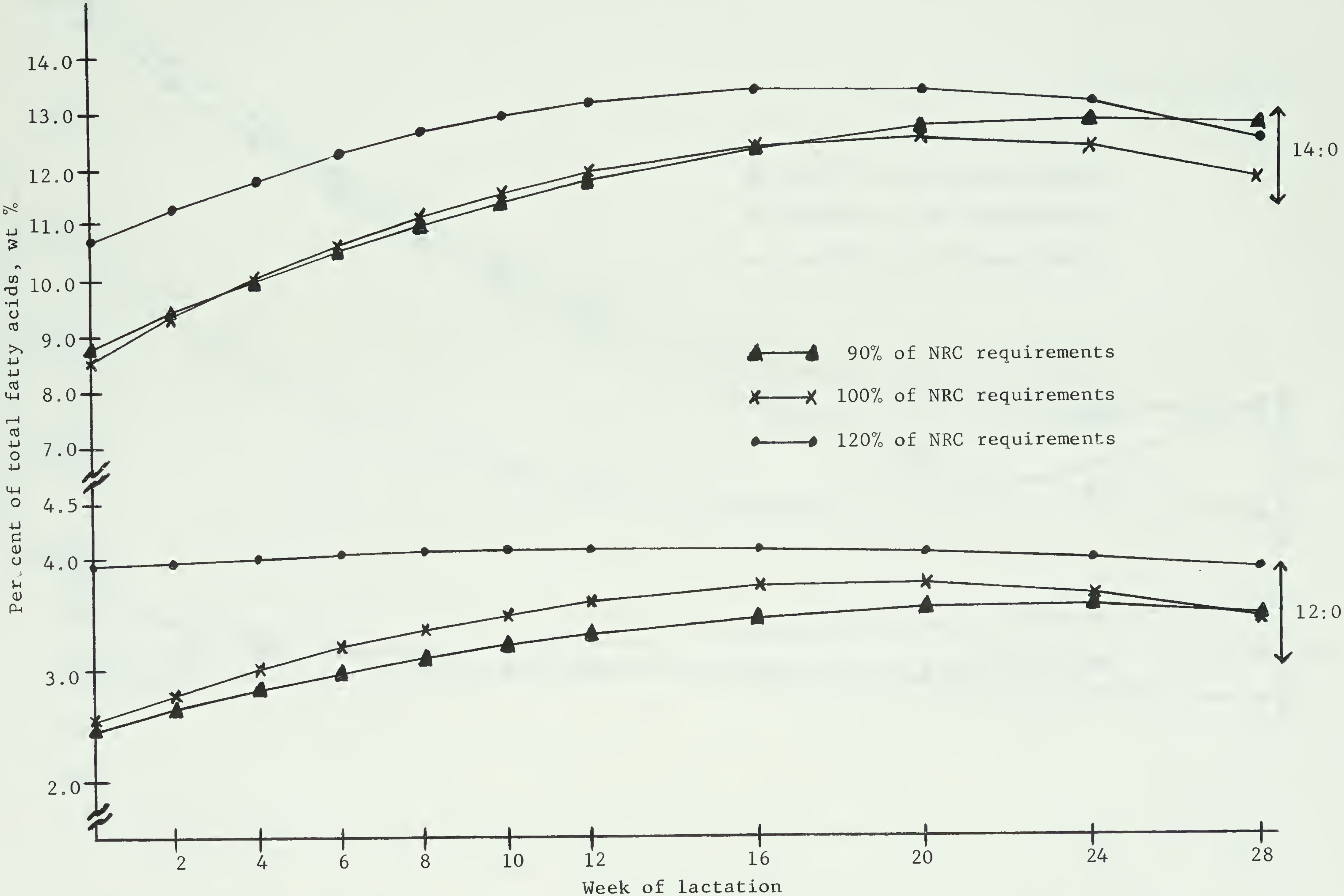

Fig. 14. The effect of level of energy in the ration on variation in percentage 12:0 and 14:0 fatty acids during the lactation.

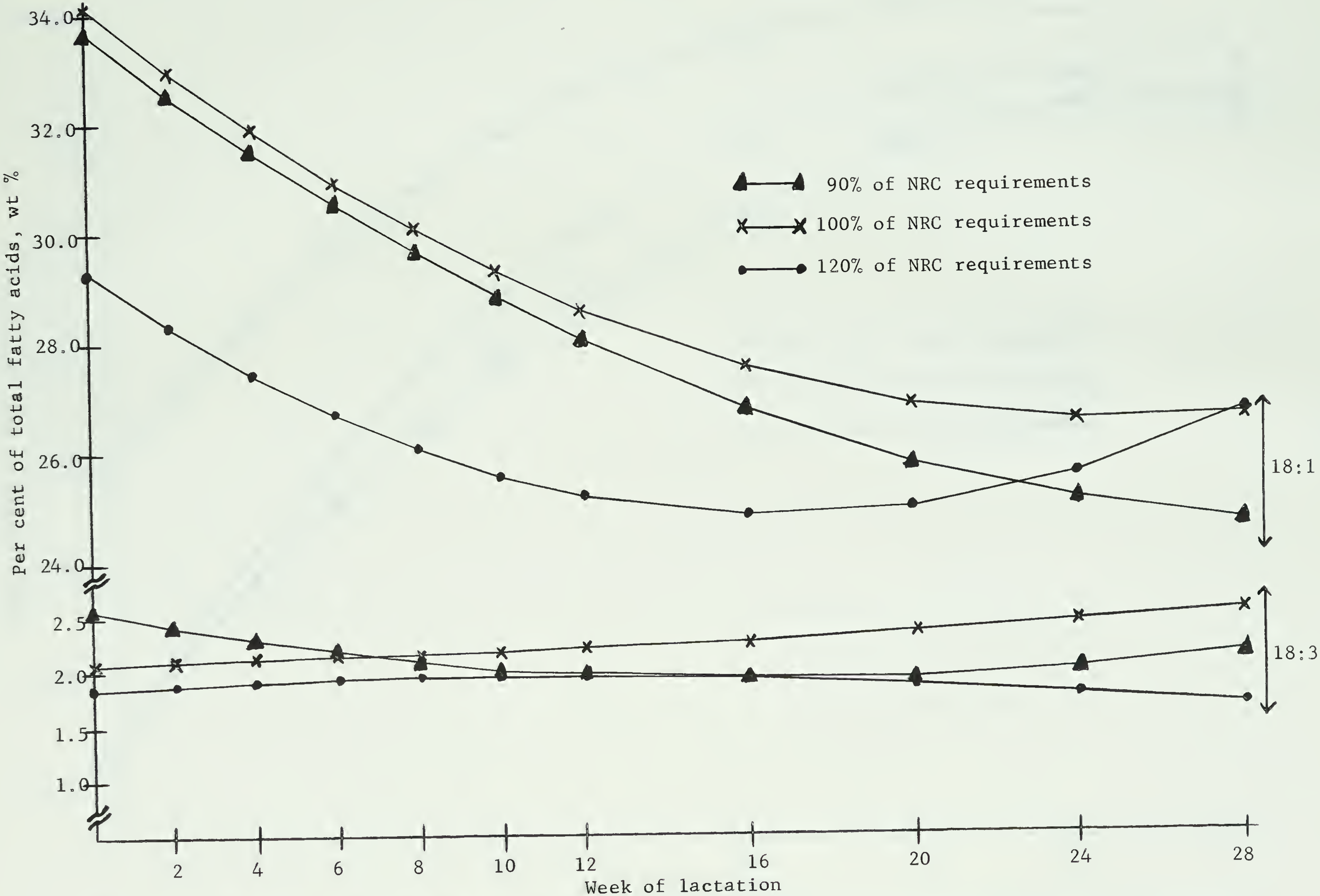

Fig. 15. The effect of level of energy in the ration on variation in percentage 18:1 and 18:3 fatty acids during the lactation.

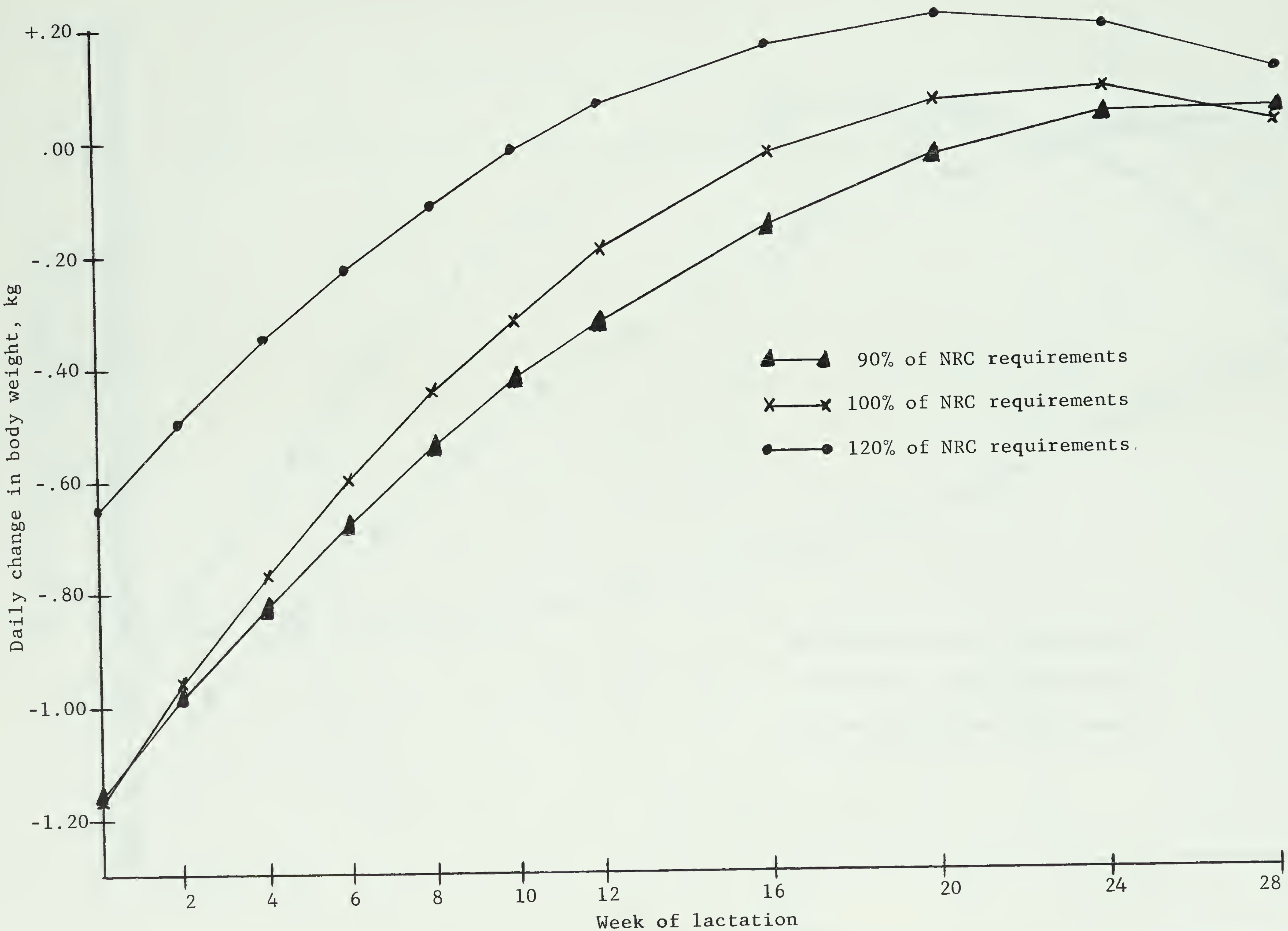

Fig. 16. The effect of level of energy in the ration on variation in body weight during the lactation.

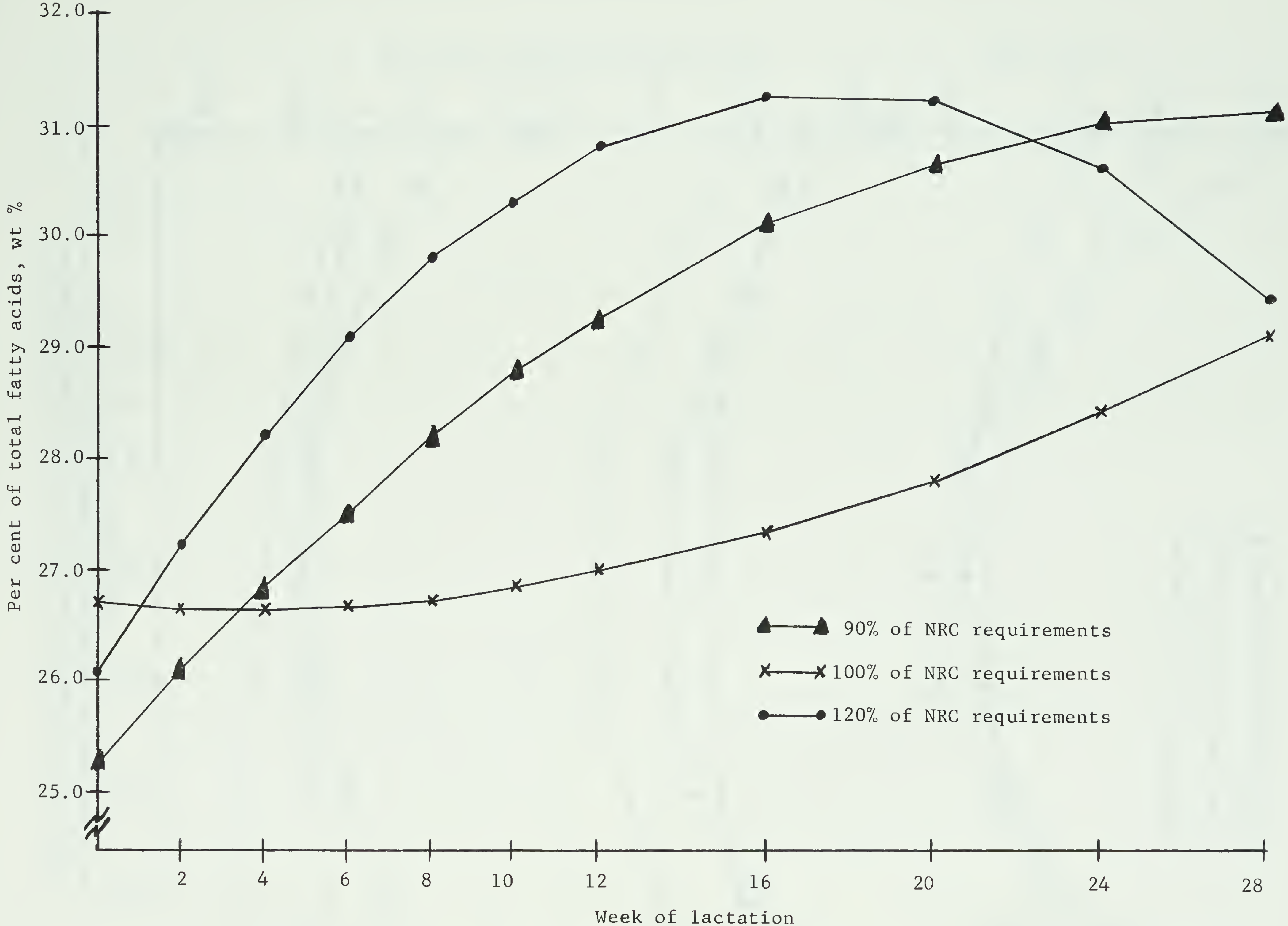

Fig. 17. The effect of level of energy in the ration on variation in percentage 16:0 fatty acid during the lactation.

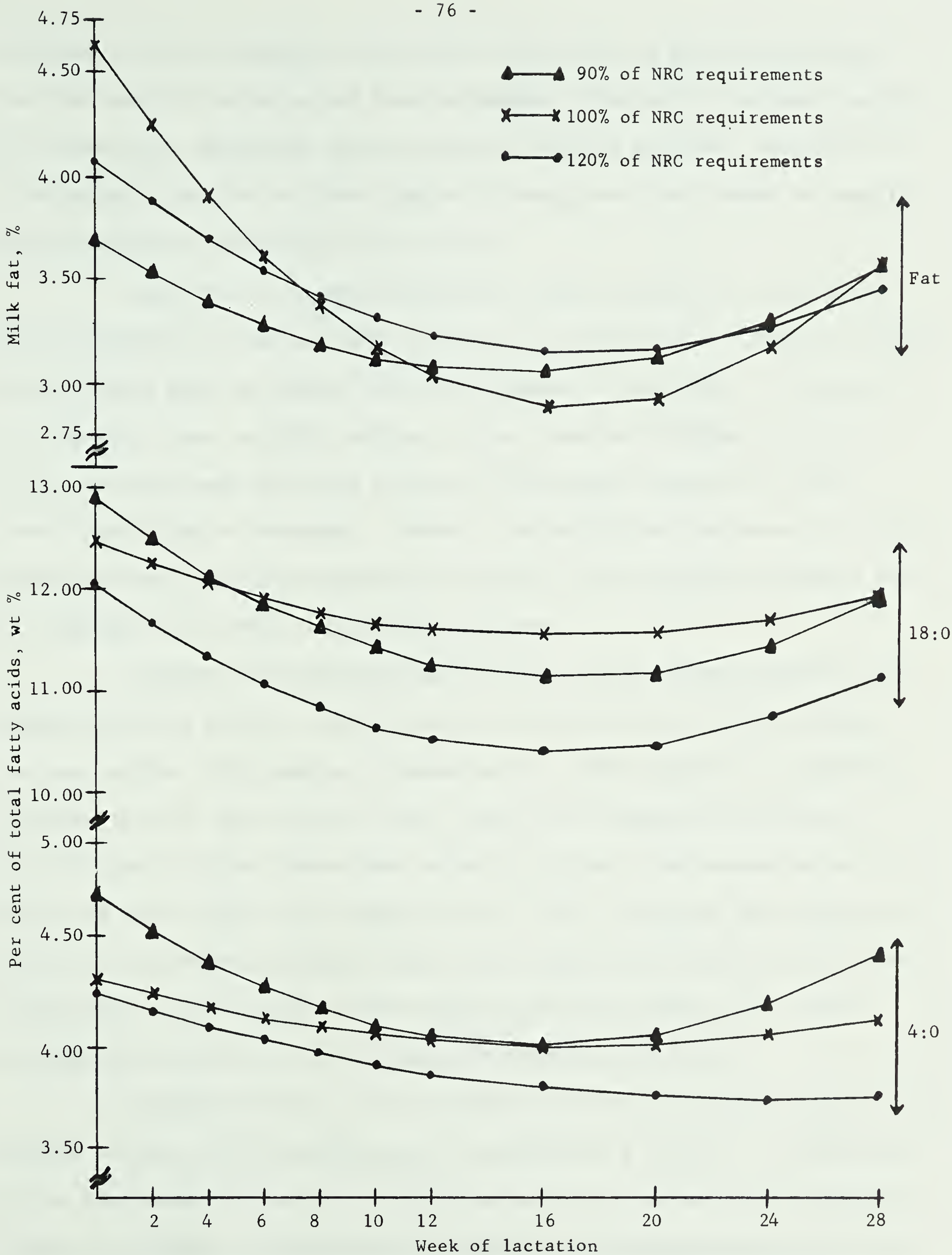

Fig. 18. The effect of level of energy in the ration on variation in percentage 4:0 and 18:0 fatty acids and per cent milk fat during the lactation.

followed a pattern similar to the level of butterfat in milk, declining to the 16th week of lactation and then increasing. Analysis of variance carried out between the regression lines of the 4:0 acid and milk fat, and the 18:0 acid and milk fat for the three levels of energy nutrition showed no significant differences between the fitted lines.

The level of roughage appeared to have less effect on the fatty acid composition of the butterfat (Table 14). The 4:0 and 6:0 acids increased significantly with the higher levels of roughage in the ration. This would be expected, since the high roughage rations resulted in higher levels of acetate in the rumen fluid and acetate is the primary precursor of short chain fatty acids in butterfat. However, the significant increase in the 18:3 fatty acid and significant decrease in the 18:2 fraction with increasing level of roughage in the ration can not be explained.

Somewhat contradictory reports have been published concerning the changes in fatty acids in milk fat when high concentrate or high roughage rations are fed. For example, Dronawat et al. (1966) measured a significant increase in 14:0, 18:1 and 18:2 fatty acids and a significant decrease in the 16:0 and 18:0 fatty acids when animals received a fat depressing ration. Beitz and Davis (1964) and Jorgensen et al. (1965) indicated that during the period of low roughage feeding, there was a significant decrease in milk fat, rumen acetate, milk fat short chain fatty acids and stearic acid, with a concomitant increase in all long chain unsaturated fatty acids.

During lactation, the 4:0 acid in butterfat followed a pattern similar to that of the percentage fat in milk (Fig. 9 and 19). It declined to the 16th week; thereafter, it increased when the cows were fed the higher levels of roughage, as concentrates composed a decreasing proportion of the total ration. The 18:3 fraction increased steadily throughout lactation

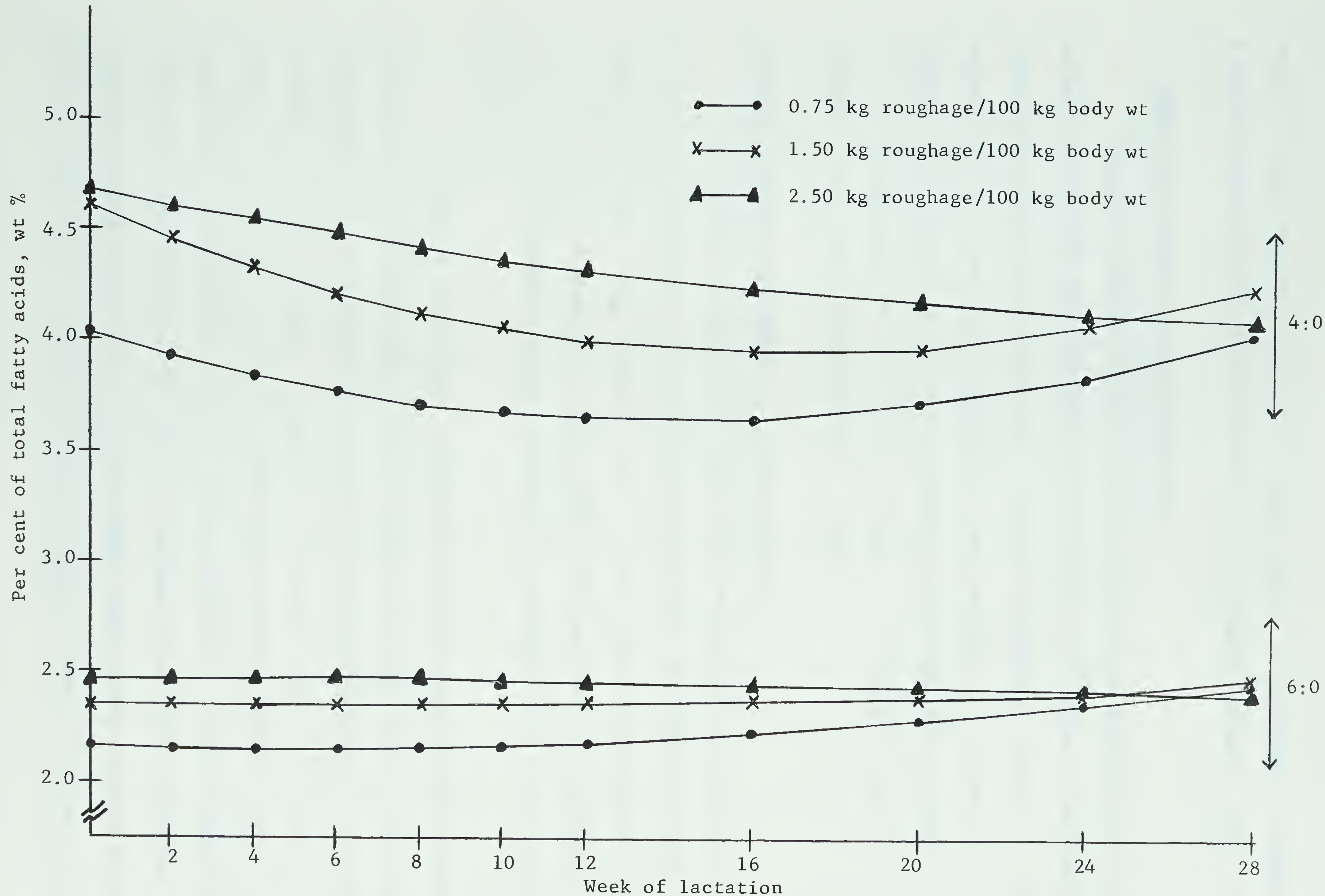

Fig. 19. The effect of level of roughage in the ration on variation in percentage 4:0 and 6:0 fatty acids during the lactation.

(Fig. 20) when cows were fed the two higher levels of roughage. In addition, a steady decline was found for the 18:2 acid throughout lactation (Fig. 20),

f. Energy utilization for milk production

Digestible energy consumed per kg of FCM and SCM produced, and net energetic efficiency were calculated for all cows at the stage of maximum milk production (period 1) and just before lactation termination (period 2). Mean values for each of the nine treatments in both periods, and average daily change in body weight for the first 28 weeks of lactation are presented in Table 15; mean values for the effects of levels of energy and levels of roughage in the ration are summarized in Table 16.

Digestible energy requirements for milk production

The intake of digestible energy above maintenance requirements of the cows was obtained by calculating the maintenance requirements, using the formula 167.2 kcal x $W_{kg}^{.75}$ (Garrett et al., 1959), and subtracting this from the total intake of digestible energy. These data was used to calculate utilization of digestible energy in excess of maintenance for production of FCM and SCM.

In period 1, the amount of digestible energy available for production of FCM and SCM increased with level of roughage with cows fed the high energy ration (Table 15), but differences were small and not consistent with the low and medium levels of energy. In period 2, there was little difference in efficiency of utilization of digestible energy between comparable groups of cows fed the low and medium levels of energy (Table 15), but more energy was utilized for FCM and SCM as the level of roughage in the ration increased. Cows fed the high energy ration had the highest requirements for milk production, but there was no consistent trend according to level of roughage in the

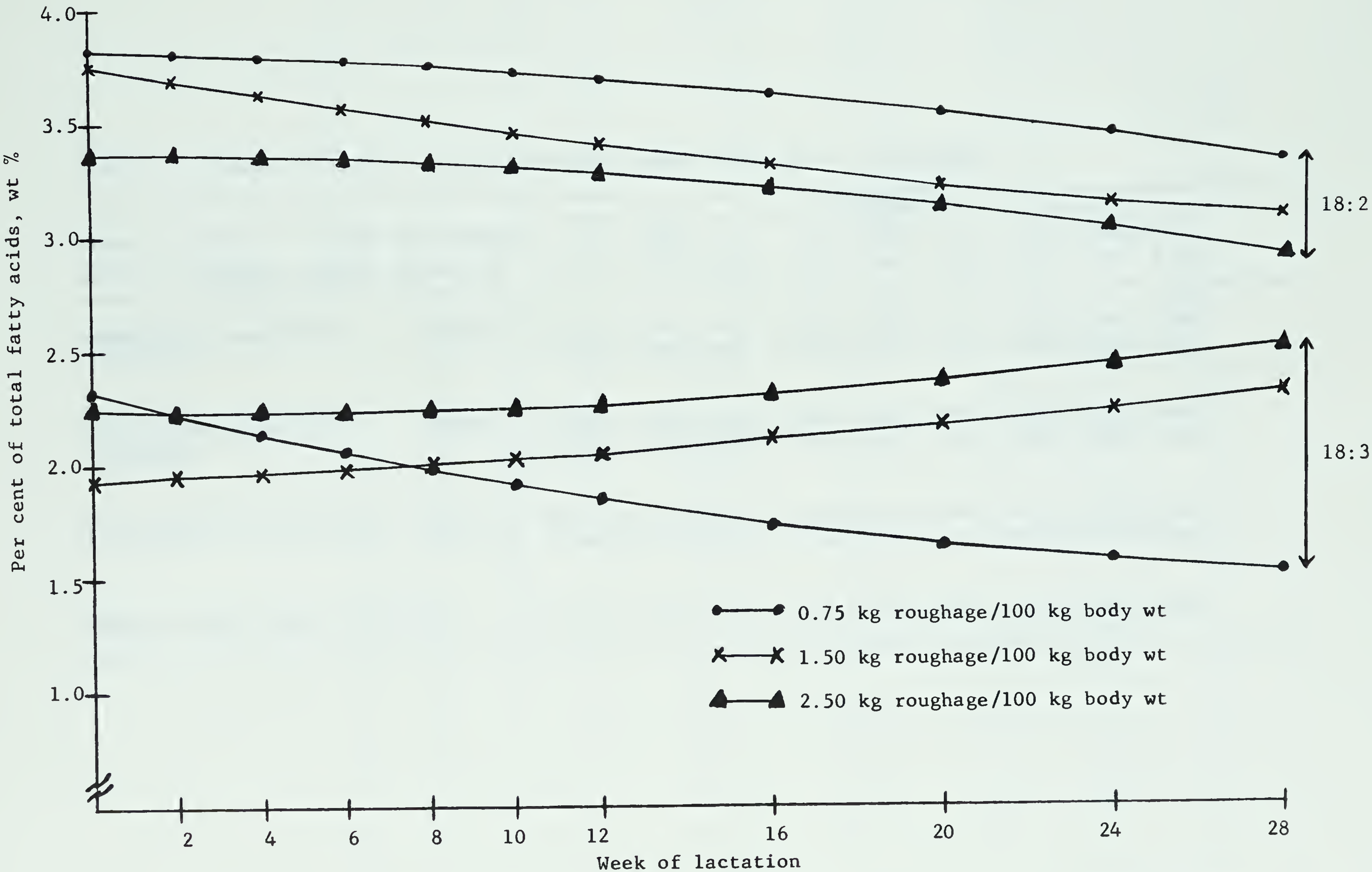

Fig. 20. The effect of level of roughage in the ration on variation in percentage 18:2 and 18:3 fatty acids during the lactation.

Table 15. Energy utilization and average body weight loss for all treatments

		Treatments								
Level of energy, % of NRC requirements		90			100			120		
Level of roughage, kg/100 kg body wt		0.75	1.50	2.50	0.75	1.50	2.50	0.75	1.50	2.50
Digestible energy/FCM,	Period 1	1.470	1.126	1.247	1.249	1.502	1.261	1.008	1.547	1.966
mcal/kg	" 2	1.115	1.424	2.107	1.176	1.532	1.770	2.166	2.593	2.221
Digestible energy/SCM,	Period 1	1.412	1.113	1.216	1.180	1.456	1.233	1.032	1.477	1.945
mcal/kg	" 2	1.112	1.435	2.069	1.153	1.525	1.746	2.087	2.556	2.185
Net energetic efficiency,%	Period 1	49.25	59.15	41.97	61.39	41.66	48.64	52.81	46.69	38.14
	" 2	77.68	62.20	43.22	77.28	66.43	51.37	45.31	37.90	38.43
Change in body weight, kg /day		-0.267	-0.266	-0.348	-0.113	-0.165	-0.468	0.017	-0.062	0.040

Table 16. Energy utilization and average body weight loss according to level of energy and roughage fed

		Level of energy, % of NRC requirements			Level of roughage, kg/100 kg body wt			Average of all cows
		90	100	120	0.75	1.50	2.50	
Digestible energy/FCM, mcal/kg	Period 1	1.281	1.337	1.507	1.242	1.392	1.491	1.375[g]
	" 2	1.549	1.493	2.327	1.486	1.850	2.033	1.790[h]
	Av	1.415[a]	1.415[a]	1.917[b]	1.364[y]	1.621	1.762[z]	
Digestible energy/SCM, mcal/kg	Period 1	1.247	1.290	1.485	1.208	1.349	1.465	1.341[g]
	" 2	1.539	1.475	2.276	1.451	1.839	2.000	1.763[h]
	Av	1.393[a]	1.383[a]	1.881[b]	1.330[y]	1.594	1.733[z]	
Net energetic efficiency, %	Period 1	50.12	50.56	45.88	54.48	49.17	42.92	48.85
	" 2	61.03	65.03	40.55	66.76	55.51	44.34	55.54
	Av	55.57[a]	57.80[a]	43.22[b]	60.62[vy]	52.34[z]	43.63[xy]	
Change in body wt, kg/day		-0.294[c]	-0.249[c]	-0.002[d]	-0.121	-0.164	-0.259	-0.182

[abcd]Values in the same row, under energy heading, with superscripts [a] and [b] are significantly different ($P < .01$); [c] and [d] ($P < .05$).

[vxyz]Values in the same row, under roughage heading, with superscripts [v] and [x] are significantly different ($P < .01$), [y] and [z] ($P < .05$).

[gh]Averages of all cows for the same variable with superscripts [g] and [h] are significantly different ($P < .05$).

ration within this group.

On the average, cows fed the medium and low levels of energy and the low and medium levels of roughage in period 1 produced milk more efficiently in terms of digestible energy consumed than cows fed the high energy and high roughage ration (Table 16). However, the differences were not large enough to be statistically significant. These results might have been expected, since all cows ate to maximum appetite and there were no appreciable differences in milk production in period 1. In period 2, there was no difference in efficiency between cows fed the low and medium levels of energy, but these were more efficient than cows fed the high level of energy. Digestible energy consumed per kg of FCM increased with increasing levels of roughage in the ration.

When the digestible energy consumed per kg of FCM was averaged for the two periods (Table 16), there was no difference between the low and medium energy groups, but this was significantly lower ($P < .01$) than that of the high energy group. Cows fed at the level of 90% of their energy requirements had less digestible energy above their maintenance requirements, but they produced less milk, lost more weight and had the same requirement of 1.4 mcal digestible energy per kg of FCM as cows fed at 100% of their energy requirements. Cows fed at 120% of their energy requirements used 1.9 mcal digestible energy per kg FCM. Their high requirements for milk production illustrates the fact that these cows were over fed and were using energy to store body fat, in addition to production of milk. Although these cows lost weight initially, they were gaining in weight after the 10th week of lactation (Fig. 16); by the 28th week of lactation they had regained nearly all of the weight lost early in lactation, since their average daily loss was only 0.002 kg (Table 16).

The average digestible energy consumed per kg of FCM during the two periods for the cows fed the three levels of roughage in the ration, showed a significantly lower value ($P < .05$) for the group fed the low level of roughage than that of the group fed the high level of roughage (Table 16). This might be expected since digestible energy in roughage is not used as efficiently for productive purposes as is the digestible energy in grain.

Data from the literature suggest variable conversion of TDN or digestible energy to FCM. Armsby (1917) and McCullough (1964) reported 0.627 lb of TDN (1.254 mcal) per kg FCM, whereas Jumah et al. (1965) found conversion to range from 1.078 to 1.452 mcal per kg of FCM for the highest and lowest levels of production, respectively. In the present study, observed values for digestible energy/kg FCM for the three levels of energy were substantially higher than those found by Jumah et al. (1965) but there was a significant trend to increasing values at lower levels of production.

Net energetic efficiency for milk production

After absorption, digestible energy is utilized in various ways. Some is lost in the urine and methane, and the remainder (metabolizable energy) is distributed between heat increment, maintenance and production (milk and/or body tissue). Reports in the literature indicate that level of intake and ration composition alter losses in the urine, methane and heat increment either proportionally or inversely to the quantity of digestible energy consumed (Blaxter, 1962; Coppock et al., 1964b; Flatt et al., 1966; Moe et al., 1965; Reid et al., 1966).

Since no measurements of these variables were carried out in this experiment, but weight changes in individual cows were measured, a calculation was made of the net energetic efficiency for milk production.

Net energetic efficiency (NEE) was calculated according to the method of Brody (1945) as follows:

$$NEE = \frac{FCM \times 748 \text{ kcal}}{\text{digestible energy intake (kcal)} - (167.2 \text{ kcal} \times W^{.75}_{kg}) \pm (9240 \text{ kcal} \times \Delta W)}$$

This method assumes an average gross caloric value of 748 kcal per kg of FCM, that 167.2 kcal x $W^{.75}_{kg}$ equals the requirement for maintenance, and that each kg in gain or loss of body weight accounts for 9240 kcal digestible energy (4.62 lb TDN x 2000 kcal per lb).

In period 1, differences were not consistent between treatments in each of the three levels of energy nutrition (Table 15). In period 2, differences between treatments were much greater and more consistent than in period 1. At each level of energy, efficiency decreased with increasing level of roughage, and was much lower in the cows fed the high level of energy than in the cows fed the low and medium levels of energy.

On the average (Table 16) net energetic efficiency in period 1 was the same in cows fed the low and medium levels of energy (50%) and slightly lower in cows fed the high level of energy (46%). There was a trend to decreasing efficiency with increasing level of roughage. Since all cows were apparently eating to full capacity in this period, it would not be expected that differences would be particularly large or consistent. Another factor which could have affected the net energetic efficiency values in the first period was the difficulty in measuring the exact body weight losses of the experimental animals. Concomitant losses of body fat and gains in body water might tend to keep body weight constant and mask the caloric value made by the energy stores (Bath et al., 1965; Flatt et al., 1965; Jumah et al., 1965, Reid, 1961).

In period 2 highest average efficiency was obtained for the group fed the medium level of energy (Table 16). As lactation progressed it appears

that this level of energy intake was adequate to sustain milk production without severe losses in liveweight. Cows on the low level of energy were slightly less efficient, lost the most body weight and could not sustain adequate levels of milk production (Fig. 8). Lowest efficiency was obtained in cows fed the high level of energy, indicating that these cows were consuming more digestible energy than they could efficiently convert into milk and were storing more body fat later in the lactation. The above data agree with the hypothesis of Van Soest (1963) who suggested that a fundamental antagonism exists between metabolism for milk production and gain in body weight.

Averages for the two periods (Table 16) show that the efficiency of cows fed the medium level of energy was slightly higher than that of cows fed the low level of energy, and both of these were significantly ($P < .01$) higher than that of cows fed the high level of energy.

An increase in the proportion of roughage in the ration resulted in a significant ($P < .01$) decrease in the net energetic efficiency for milk production (Table 16). While in many reports it has been noted that proportions of concentrates between 20 and 67% of the ration did not change the efficiency for milk production (Coppock et al., 1964a,b; Elliot and Loosli, 1959; Flatt et al., 1966), other reports have indicated a beneficial effect of higher levels of concentrates in the ration up to the point at which the milk fat test began to be depressed (Van Soest, 1963; Baumgardt, 1967). Although, the average per cent milk fat obtained in this experiment significantly decreased (Table 10) when the proportion of concentrate in the ration increased from 13 to 68%, the extent of the depression could conceivably have been within the limits where net energetic efficiency for milk production would not be decreased.

The average net energetic efficiency for FCM production was lower during the first period, as compared to the second period. This was due mainly to the increase in net energetic efficiency for the animals fed the low and medium levels of roughage in the rations with the low and medium levels of energy during the latter stages of lactation (Table 15). On the contrary, Jumah et al. (1965) found that the net energetic efficiency decreased by a significant margin from the first until the seventh month. Inability to accurately measure change in body weight and the energy supplied by loss in body weight would affect net energetic efficiency for milk production. This may have been responsible, in part at least, for the increase in net energetic efficiency late in lactation found in this experiment, as compared with the decrease obtained by Jumah et al. (1965).

GENERAL DISCUSSION AND SUMMARY

Experimental rations containing three levels of roughage,0.75, 1.50 and 2.50 kg per 100 kg of body weight, were fed to lactating Holstein-Friesian cows at 90, 100 and 120% of NAS-NRC (1966) requirements for digestible energy. The experimental period lasted for 28 to 44 weeks after calving. Dry matter and gross energy intake, apparent digestion coefficients of dry matter, crude protein and gross energy, rumen VFA and pH,and energetic efficiency for milk production were measured at maximum milk production (period 1) and just before lactation termination (period 2). Milk production, milk composition and body weight changes were measured during the first 28 weeks of lactation.

The level of energy and level of roughage in the experimental rations had small and inconsistent effects on the amounts of dry matter and gross energy consumed by all cows at maximum milk production (period 1). This was caused primarily by the inability of the cows to consume their daily ration, with the exception of two animals in the low energy group. Under the conditions imposed in this study, it would appear that lactating cows were unable to consume feed above the level of 90% of NRC requirements during the peak of milk production.

It was found throughout the experimental period that the animals fed the high energy rations never consumed their daily ration allotments, whereas animals in the medium and low energy groups consumed theirs after approximately 17 and 14 weeks, respectively.

During the latter stages of the lactation (period 2) dry matter and gross energy intakes were greater as the level of energy in the ration increased. This was caused by the larger allotments of feed for the higher energy groups and the fact that milk production decreased faster for the

low energy group during the latter part of lactation. The intake of dry matter and gross energy during the second period increased when the proportion of roughage in the ration increased, which was the result of the lower digestible energy content of roughage as compared to concentrate.

Level of energy or of roughage in the ration did not significantly affect the apparent digestion coefficients of dry matter, crude protein and gross energy within periods 1 and 2. The non-significant differences for these apparent digestion coefficients were expected during maximum milk production because of the small differences in dry matter intake during this period. However, differences between the apparent digestion coefficients were expected during the latter stages of lactation, since cows fed the high level of energy were consuming 60.9 mcal of gross energy as compared to 49.7 mcal by cows fed the low level of energy, and roughage:concentrate ratios in the second period decreased from 8.8 at the low level of energy to 1.9 at the high level of energy. Since the proportion of roughage was higher in rations fed to cows on the low level of energy, this might have offset any trend to increased digestibility that might have been expected as a result of the lower feed intake. However, during this period, increasing proportions of roughage did not affect apparent digestion coefficients within any of the three levels of energy. Therefore, it appears that varying the proportions of roughage in the ration did not have marked effects on digestibility.

A significant difference was obtained for the apparent digestion coefficients of dry matter, crude protein and gross energy between periods 1 and 2; with the lower coefficients measured during the second period. The actual cause of this depression in digestibility is difficult to determine. Although decreasing dry matter intake and increasing proportions of

roughage in the ration as the lactation progressed might have affected digestibility, neither of these variables affected digestibility within period 2. Therefore, it seems evident from these experimental results that, in addition to the effects of level of intake and ration composition on digestibility (Flatt et al., 1966; Moe et al., 1965; Putnam and Loosli, 1959), other physiological factors may be involved.

Average molar concentrations of total VFA in rumen liquid for all animals decreased as the lactation progressed which may have been caused by a decrease in the gross energy intake in period 2 as compared to period 1. The average pH of rumen contents of all animals increased from 6.52 at the peak of milk production to 6.83 before cessation of the experimental period. This showed that the rumen pH decreased as the total amount of VFA in the rumen liquor increased.

Level of energy in the ration did not affect the molar proportions of acetate, propionate, n-butyrate and n-valerate in the rumen liquor. A significant difference, which at the present time seems of limited importance, was obtained for iso-butyrate and iso-valerate when the cows were fed rations at different levels of energy. Increasing the proportion of roughage in the ration resulted in a significant increase in the molar proportion of acetate and a decrease in the molar proportion of propionate. Balch et al. (1955), Shaw et al. (1959) and Stanley et al. (1964) noted similar effects on the acetate and propionate production in the rumen. The average molar proportions of iso-butyrate, n-butyrate, iso-valerate and n-valerate in this study varied between levels of roughage in the ration, but no significant differences were detected.

No significant differences were noted in the average milk, FCM and SCM production over the first 28 weeks of lactation between cows fed the

different levels of energy or roughage. However, a significant decrease was noted after the 16th week for milk produced by the group fed the low level of energy as compared to the medium and high energy groups. It would appear that cows fed the low level of energy were able to draw on reserves of body fat early in the lactation to achieve high levels of production. Body reserves were rapidly depleted and the intake of dietary nutrients by cows fed energy at 90% of NRC requirements was insufficient to maintain milk production. This was further emphasized by the fact that cows milked for only 34 weeks when fed the low level of energy as compared to 41 weeks when fed the medium and high levels of energy.

Increasing the level of roughage in the ration from 0.75 or 1.50 kg to 2.50 kg per 100 kg of liveweight resulted in a significantly higher percentage of milk fat. Similar results have been reported by Emery et al. (1964) and Leighton (1965). The phenomenon of milk fat depression has been found to be related to changes in the proportion of roughage in the ration, and to alterations in the proportions of VFA in rumen contents (Beitz and Davis, 1964). However, there is considerable uncertainty concerning the actual relationship between the percentage of milk fat and the proportions of individual volatile fatty acids in the rumen.

The percentage milk protein increased significantly as the level of energy in the ration increased. This agrees with the results of Becker et al. (1965) who after reducing the TDN intake by 25% noted a 10% decline in the milk protein content. A significant change in per cent milk protein was also observed in the present study between cows fed different levels of roughage. However, the reason for this effect was not apparent, since the lowest value for milk protein was obtained with cows fed the medium level of roughage in the ration.

The percentage SNF in the milk increased when the level of energy in the ration increased from 90% to either 100 or 120% of NRC requirements. Several reports in the literature noted a similar effect (Balch et al., 1961; Holmes et al., 1960),whereas other reports have found no beneficial effect by increasing the energy intake (Bernett and Olson, 1963; Brown et al., 1962b).

The major components of butterfat were 14:0, 16:0, 18:0 and 18:1 fatty acids with average percentages between 11.4 and 29.0 of the total fatty acids measured. The 4:0, 6:0, 8:0, 10:0, 12:0, 18:2 and 18:3 fatty acids were present in amounts varying from 1.3 to 4.4%. The higher levels of energy in the ration in this study were associated with significant increases in the 8:0, 10:0, 12:0 and 14:0 acids, whereas the 18:0, 18:1 and 18:3 fatty acids were significantly lower. The variation obtained in these acids may be attributable to the much higher body weight losses by the animals fed the low and medium levels of energy in the ration as compared to the high energy group. A loss in body weight would have been associated with mobilization of body fat, resulting in a comparative increase in the 16:0, 18:0 and 18:1 acids in the blood stream. This would provide an opportunity for more of these acids to be incorporated into milk fat, and would thus account for the higher levels of these acids in butterfat from cows fed the low level of energy. In general, there was a greater incorporation of long chain fatty acids into milk fat during the first half of lactation, when more body weight was lost as a result of feeding experimental rations which contained less energy.

An increase in the level of roughage in the ration significantly increased the 4:0 and 6:0 acids. This was expected, since the high roughage rations resulted in higher levels of acetate in rumen fluid, and acetate is

the primary precursor of short chain fatty acids in butterfat. A significant increase was also noted in the 18:3 fraction and a significant decrease in the 18:2 acid with increasing level of roughage in the ration.

On the average, cows at maximum milk production fed the low and medium levels of energy and the low and medium levels of roughage produced milk more efficiently, in terms of the digestible energy consumed above maintenance per kg of FCM, than cows fed the high energy and high roughage rations. In period 2, cows fed the high energy ration had the highest conversion for milk production, and there was little difference in the efficiency of utilization of digestible energy between cows fed the low and medium levels of energy.

When results for the two periods were averaged, cows fed the low and medium levels of energy had the same conversion of digestible energy per kg of FCM, and this was significantly lower than that of cows fed the high level of energy. A similar analysis carried out between cows fed the three levels of roughage showed that energy utilization per unit of milk produced increased with each increase in level of roughage in the ration. This might be expected since digestible energy in roughage is not used as efficiently for productive purposes as is the digestible energy in grain.

Net energetic efficiency, as calculated according to the method of Brody (1945), was slightly higher at maximum milk production for the cows fed the low and medium energy rations as compared to the high energy group. During this period there was also a trend to decreasing efficiency with increasing level of roughage in the experimental ration. Since all cows were apparently eating to maximum appetite in this period, it was not expected that differences would be particularly large or consistent. In period 2, similar effects were noted but differences between treatments were much greater and more

consistent than in period 1. When data from the two periods were averaged, there was little difference in net energetic efficiency between cows fed energy at 90 or 100% of NRC requirements, but this was significantly higher than that of cows fed energy at 120% of NRC requirements.

In the early part of lactation cows were unable to consume the experimental rations above the level of 90% of NRC requirements, and all groups produced milk at about the same average level. Consequently, this level of feeding appeared to be adequate until approximately the twelfth week of lactation. After this period, the cows were able to consume energy up to 100% of NRC requirements; when the level of feeding was not increased above 90% of NRC requirements, milk production declined faster and the cows had shorter lactations than when cows were fed at the higher levels of energy. When cows were fed energy above the level of 100% of NRC requirements, there was no increase in milk production and there was a decrease in efficiency of utilization of feed for milk production.

In conclusion, it seemed that maximum milk production could be obtained by feeding rations to provide energy at 90% of NRC requirements during the peak of lactation. As production began to decline, and the daily ration decreased to the extent that the cow could consume energy up to 100% of NRC requirements, this increase in the level of feeding would be required to maintain persistency in the lactation.

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APPENDIX

Gas-Liquid Chromatography Methods

Volatile fatty acids in rumen fluid

A 4 μl sample of the stored aqueous rumen fluid was injected directly into a column (3 mm x 1.5 m) packed with a commercial preparation of 5% FFAP on Porapak Q. Helium, hydrogen and air were supplied to the detector at a flow rate of 75 ml/min, 40 ml/min and 40 ml/min, respectively. An injection temperature of 215°C and an oven temperature of 205°C were used throughout. A flame setting of 1 was maintained and attenuations of 2, 4, 8 and 16 were used as required. The output was fed to a Minneapolis Honeywell strip chart recorder with a full-scale deflection of 1 millivolt.

A standard set of volatile fatty acid solutions, containing acetate, propionate, iso-butyrate, n-butyrate, iso-valerate and n-valerate, was prepared and analyzed as described. Peak heights of the sample volatile fatty acids were compared to the standard volatile fatty acids and peak height ratios used in determining the unknown amount of each of the volatile fatty acids in the rumen fluid.

Fatty acids in milk fat

To measure the fatty acids present in the methanolic ester solution, the freeze drying bulb was opened and a 8 μl sample of the contents injected directly into a column (5 mm x 3 m) packed with 20% (w/w) diethylene glycol succinate (DEGS) on 60/80 mesh firebrick. Helium carrier gas was used at a flow rate of 100 ml/min. The column was programmed linearly with a model 326 Aerograph from 70° to 225°C at a rate of 8°C per minute. An injection port temperature of 240°C and a detector temperature of 250°C were used throughout. A filament current of 200 milliamps was maintained and attenuations of 2, 4, 8 and 16 were used as required.

A model 44 Microcord recorder was used at an input setting of 1 millivolt and a chart speed of 1" per minute. To measure peak areas a model 49 Integraph Automatic Integrator was attached to the recorder and digits marked on the baseline under each peak of the chromatograph were counted. To calculate weight per cent of the fatty acids, correction factors (Appendix Table 1) were applied to digital peak count figures and the corrected figures were converted to weight per cent by taking them as a per cent of total digits of all peaks on the chromatogram. Peaks were identified by 'spiking' esterified fat samples with pure standard fatty acid esters and by a semilogarithmic plot of carbon number versus logarithm of the retention time. Correction factors (Appendix Table 1) for the conversion of peak areas to weight percentage of methyl esters were established by measuring mixtures of pure compounds for their recovery in the manner as described.

Appendix Table 1. Correction factors for the conversion of digital peak count data from chromatograms to weight percentage methyl esters

Fatty acid component	Correction factors from Smith (1961)	Correction factors from DeMan (1964)	Present study
4:0	1.07	.74	.76
6:0	.84	.77	.76
8:0	.86	.81	.81
10:0	.87	.86	.86
12:0	.79	.91	.90
14:0	.92	.96	.96
16:0	1.02	1.01	1.00
18:0	1.08	1.06	1.06
18:1	1.08	1.10	1.10
18:2	1.10	1.15	1.24
18:3	1.17	1.20	1.33

Appendix Table 2. Regression equations expressing the variation in percentage milk fat, milk protein and milk SNF with stage of lactation

Item (y)	Regression equation	R^2	N	r_{ty}	F
Milk fat, %					
0.75 kg roughage/ 100 kg body wt	$y=4.057-0.155\ T + 0.0050\ T^2$ (0.041)[a] (0.0013)	0.38	90	-0.07	7.34
1.5 kg roughage/ 100 kg body wt	$y=4.135-0.152\ T + 0.0047\ T^2$ (0.033) (0.0011)	0.44	90	-0.17	10.69
2.5 kg roughage/ 100 kg body wt	$y=4.198-0.091\ T + 0.0024\ T^2$ (0.029) (0.0010)	0.38	90	-0.29	7.45
Milk protein, %					
90% NRC	$y=3.863-0.092\ T + 0.0026\ T^2$ (0.017) (0.0006)	0.55	90	-0.36	19.03
100% NRC	$y=3.919-0.067\ T + 0.0017\ T^2$ (0.018) (0.0006)	0.49	90	-0.41	13.53
120% NRC	$y=4.090-0.062\ T + 0.0017\ T^2$ (0.021) (0.0007)	0.35	90	-0.24	6.11
Milk protein, %					
0.75 kg roughage/ 100 kg body wt	$y=3.752-0.030\ T + 0.0007\ T^2$ (0.019) (0.0006)	0.25	90	-0.22	2.95
1.5 kg roughage/ 100 kg body wt	$y=4.011-0.095\ T + 0.0025\ T^2$ (0.019) (0.0006)	0.55	90	-0.41	19.22
2.5 kg roughage/ 100 kg body wt	$y=4.108-0.095\ T + 0.0027\ T^2$ (0.023) (0.0007)	0.45	90	-0.29	11.17
Milk SNF, %					
90% NRC	$y=9.301-0.074\ T + 0.0022\ T^2$ (0.019) (0.0006)	0.40	90	-0.21	8.36
100% NRC	$y=9.439-0.044\ T + 0.0009\ T^2$ (0.022) (0.0007)	0.36	90	-0.34	6.67
120% NRC	$y=9.528-0.071\ T + 0.0020\ T^2$ (0.027) (0.0009)	0.30	90	-0.18	8.36

[a]Values in parentheses refer to standard error of regression coefficient for value above.

Appendix Table 3. Regression equations expressing the variation in percentage of 8:0, 10:0, 12:0 and 14:0 milk fatty acids with stage of lactation

Item (y)	Regression equation	R^2	N	r_{ty}	F
8:0, wt %					
90% NRC	$y=1.288+0.013\ T - 0.0002\ T^2$	0.20	90	0.19	1.82
	(0.015)[a] (0.0005)				
100% NRC	$y=1.246+0.036\ T - 0.0010\ T^2$	0.23	90	0.17	2.44
	(0.019) (0.0006)				
120% NRC	$y=1.616-0.008\ T + 0.0001\ T^2$	0.12	90	-0.12	0.66
	(0.019) (0.0006)				
10:0, wt %					
90% NRC	$y=2.367+0.080\ T - 0.0018\ T^2$	0.38	90	0.34	7.25
	(0.033) (0.0011)				
100% NRC	$y=2.493+0.106\ T - 0.0030\ T^2$	0.33	90	0.20	5.22
	(0.036) (0.0012)				
120% NRC	$y=3.737-0.027\ T - 0.0006\ T^2$	0.12	90	-0.11	0.68
	(0.040) (0.0013)				
12:0, wt %					
90% NRC	$y=2.458+0.099\ T - 0.0022\ T^2$	0.42	90	0.38	9.35
	(0.037) (0.0012)				
100% NRC	$y=2.539+0.131\ T - 0.0035\ T^2$	0.43	90	0.31	10.10
	(0.034) (0.0011)				
120% NRC	$y=3.930+0.021\ T - 0.0008\ T^2$	0.06	90	-0.03	0.19
	(0.044) (0.0014)				
14:0, wt %					
90% NRC	$y=8.792+0.328\ T - 0.0067\ T^2$	0.67	90	0.63	34.82
	(0.070) (0.0023)				
100% NRC	$y=8.564+0.403\ T - 0.0089\ T^2$	0.61	90	0.48	25.14
	(0.073) (0.0024)				
120% NRC	$y=10.669+0.313\ T - 0.0089\ T^2$	0.37	90	0.22	6.73
	(0.093) (0.0030)				

[a]Values in parentheses refer to standard error of regression coefficient for value above.

Appendix Table 4. Regression equations expressing the variation in percentage of 16:0, 18:1 and 18:3 milk fatty acids and change in body weight with stage of lactation

Item (y)	Regression equation	R^2	N	r_{ty}	F
16:0, wt %					
90% NRC	$y=25.268+0.430\ T - 0.0079\ T^2$ (0.120)[a] (0.0039)	0.62	90	0.59	26.67
100% NRC	$y=26.660-0.014\ T + 0.0037\ T^2$ (0.117) (0.0038)	0.36	90	0.35	6.43
120% NRC	$y=26.063-0.602\ T - 0.0172\ T^2$ (0.182) (0.0060)	0.36	90	0.22	6.48
18:1, wt %					
90% NRC	$y=33.676-0.579\ T + 0.0092\ T^2$ (0.206) (0.0068)	0.57	90	-0.56	21.01
100% NRC	$y=34.143-0.612\ T + 0.0122\ T^2$ (0.215) (0.0071)	0.49	90	-0.46	13.64
120% NRC	$y=29.282-0.528\ T + 0.0156\ T^2$ (0.247) (0.0081)	0.23	90	-0.12	2.51
18:3, wt %					
90% NRC	$y=2.581-0.078\ T + 0.0022\ T^2$ (0.046) (0.0015)	0.19	90	-0.11	1.69
100% NRC	$y=2.075+0.009\ T + 0.0003\ T^2$ (0.043) (0.0014)	0.18	90	0.18	1.41
120% NRC	$y=1.839+0.022\ T - 0.0010\ T^2$ (0.044) (0.0014)	0.11	90	-0.08	0.54
Change in body wt, kg					
90% NRC	$y=-1.151+0.089\ T - 0.0017\ T^2$ (0.038) (0.0013)	0.45	90	0.43	10.81
100% NRC	$y=-1.175+0.110\ T - 0.0024\ T^2$ (0.051) (0.0017)	0.34	90	0.31	5.78
120% NRC	$y=-0.653+0.083\ T - 0.0020\ T^2$ (0.054) (0.0018)	0.23	90	0.19	2.38

[a]Values in parentheses refer to standard error of regression coefficient for value above.

Appendix Table 5. Regression equations expressing the variation in percentage milk fat and the percentage of 4:0 and 18:0 milk fatty acids with stage of lactation

Item (y)	Regression equation	R^2	N	r_{ty}	F
Milk fat, %					
90% NRC	$y=3.710-0.089\ T + 0.0030\ T^2$	0.36	90	0.03	6.32
	(0.026)[a] (0.0009)				
100% NRC	$y=4.632-0.205\ T + 0.0060\ T^2$	0.53	90	-0.28	17.34
	(0.037) (0.0012)				
120% NRC	$y=4.047-0.105\ T + 0.0030\ T^2$	0.28	90	-0.16	3.80
	(0.041) (0.0013)				
4:0, wt %					
90% NRC	$y=4.746-0.093\ T + 0.0029\ T^2$	0.26	90	-0.07	3.22
	(0.036) (0.0012)				
100% NRC	$y=4.332-0.039\ T + 0.0011\ T^2$	0.08	90	-0.04	0.31
	(0.052) (0.0017)				
120% NRC	$y=4.247-0.042\ T + 0.0009\ T^2$	0.17	90	-0.16	1.25
	(0.047) (0.0015)				
18:0, wt %					
90% NRC	$y=12.898-0.210\ T + 0.0062\ T^2$	0.22	90	-0.11	2.10
	(0.106) (0.0035)				
100% NRC	$y=12.458-0.109\ T + 0.0033\ T^2$	0.15	90	-0.07	1.03
	(0.079) (0.0026)				
120% NRC	$y=12.041-0.201\ T + 0.0061\ T^2$	0.18	90	-0.07	1.38
	(0.123) (0.0040)				

[a]Values in parentheses refer to standard error of regression coefficient for value above.

Appendix Table 6. Regression equations expressing the variation in percentage of 4:0, 6:0, 18:2 and 18:3 milk fatty acids with stage of lactation.

Item (y)	Regression equation	R^2	N	r_{ty}	F
4:0, wt %					
0.75 kg roughage/ 100 kg body wt	$y=4.029-0.056\ T + 0.0020\ T^2$ (0.048)[a] (0.0016)	0.22	90	-0.21	2.12
1.50 kg roughage/ 100 kg body wt	$y=4.607-0.079\ T + 0.0023\ T^2$ (0.042) (0.0014)	0.21	90	-0.10	1.95
2.50 kg roughage/ 100 kg body wt	$y=4.690-0.038\ T + 0.0006\ T^2$ (0.043) (0.0014)	0.14	90	-0.02	0.80
6:0, wt %					
0.75 kg roughage/ 100 kg body wt	$y=2.160-0.003\ T + 0.0005\ T^2$ (0.028) (0.0009)	0.18	90	0.17	1.44
1.50 kg roughage/ 100 kg body wt	$y=2.354-0.002\ T + 0.0002\ T^2$ (0.024) (0.0008)	0.07	90	0.06	0.21
2.50 kg roughage/ 100 kg body wt	$y=2.478-0.001\ T + 0.0001\ T^2$ (0.024) (0.0008)	0.06	90	-0.05	0.17
18:2, wt %					
0.75 kg roughage/ 100 kg body wt	$y=3.818-0.005\ T - 0.0005\ T^2$ (0.068) (0.0022)	0.12	90	-0.12	0.69
1.50 kg roughage/ 100 kg body wt	$y=3.759-0.035\ T + 0.0004\ T^2$ (0.052) (0.0017)	0.21	90	-0.20	1.91
2.50 kg roughage/ 100 kg body wt	$y=3.374-0.001\ T - 0.0005\ T^2$ (0.054) (0.0018)	0.15	90	-0.15	1.03
18:3, wt %					
0.75 kg roughage/ 100 kg body wt	$y=2.311-0.047\ T + 0.0007\ T^2$ (0.045) (0.0015)	0.27	90	-0.27	3.43
1.50 kg roughage/ 100 kg body wt	$y=1.933+0.006\ T + 0.0003\ T^2$ (0.041) (0.0013)	0.15	90	0.15	1.06
2.50 kg roughage/ 100 kg body wt	$y=2.252-0.005\ T + 0.0005\ T^2$ (0.044) (0.0015)	0.12	90	0.11	0.59

[a]Values in parentheses refer to standard error of regression coefficient for value above.